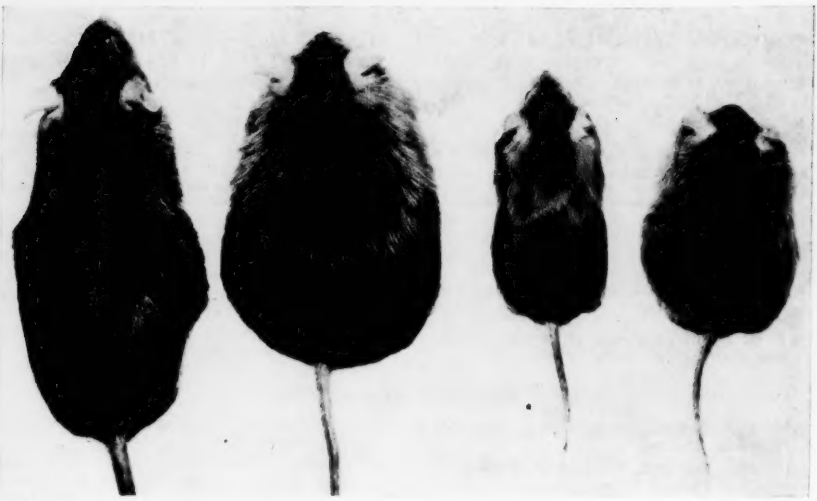
The Journal of HEREDITY



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Brain Hernia in Mice

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Coat Color in Water Buffalos

Epistasis — What Is It?

Lethality in Mexican Axolotl

Breeding Cycles in Hybrid Dogs

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The American Genetic Association

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TYPICAL FEMALES

Figure 1

Cocker spaniel female (above) and basenji female (below).

THE INHERITANCE OF ANNUAL BREEDING CYCYES IN HYBRID BASENJI-COCKER SPANIEL DOGS

J. P. Scott, John L. Fuller and John A. King*

THE wolf has an annual seasonal breeding cycle in the spring[4] but in most breeds of domestic dogs the females come into estrus at approximately six-month periods at any time of the year[1]. In contrast to that of other dogs, the cycle of the basenji breed in northern latitudes takes place in the autumn. The breed originally came from equatorial Africa, and Fuller[2] has shown that the breeding cycle can be advanced by artificially simulating declining day length during the spring and summer. The following results concern the inheritance of the latter two types of cycles in a cross between African basenjis and American cocker spaniels.

Materials and Methods

Basenjis from a single mating were reciprocally crossed with cocker spaniels of two closely related matings, and F_1 males crossed back to their purebred mothers to produce the backcross or Bx generation. The same F_1 males were then crossed with their sisters to produce two F_2 populations.

All females in the hybrid generations were kept until they showed at least two estrus periods, although in a few cases the second period was not observed because of death or because the animals were prematurely discarded by error. Small numbers of females in the parental and F_1 generations were kept for several years, and the records of five animals in each group examined in detail.

The first definite external sign of estrus in the bitch is slight bleeding from the vagina, accompanied by gradual enlargement of the vulva. The period between these first signs and the stage of receptivity is relatively short in cockers (usually about one week) and relatively long in basenjis (two weeks or more).

The results below are based on weekly examinations done by the authors, and the basic data are the dates on which bleeding from the vagina was first observed. Since bleeding could have started between examinations, this means that a maximum error of six days was possible; bleeding could have started from one to six days earlier. In a few cases, bleeding was not observed but puppies were born. The corresponding time was then estimated by counting back 63 days and adding one week. These cases are, of course, subject to slightly greater error. Any cases in which the records were doubtful were omitted from the data.

Results

Estrus in basenjis usually appears in September (Figure 2). The first estrus in all cases observed began in September or October. If we look at the subsequent estrus periods of five basenji females which were direct ancestors of the hybrids, including four females used in the cross plus their mother (Figure 3), we find that the range is extended into late August, with one case in November. We can conclude that the modal month for estrus in basenjis is September.

The estrus periods of cocker spaniels are, in contrast, scattered over many months and may occur in any month of the year (Figure 2). There is an apparent concentration of first estrus periods in the spring, but this is probably an artificial result of selecting animals born at a particular period of the year for experimental use. However, if the estrus periods of the three

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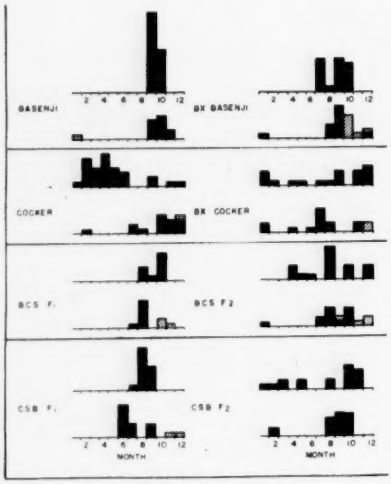


ESTRUS CYCLES

Figure 2

A—Month in which bleeding first appeared in pure bred and hybrid populations (1 = January, 2 = February, etc.). The upper line represents the first estrus cycle and the lower the second, in each population. The numbers of animals in which second estrus was observed is somewhat smaller, particularly in pure breeds. Cross-hatched columns represent instances of animals which ran two cycles less than four months apart.

females chiefly used in the cross plus their mother and grandmother are considered (Figure 3*B*), there is a slight excess of estrus periods in the spring. When individual animals are considered (Figure 4.*1*), we see that some are highly variable and some quite consistent, with a tendency for two groups of periods to appear approximately six months apart. In this small sample these peaks occur anywhere from February to May in the first part of the year, and from August to November in the latter part. There may be a slight remnant of the spring estrus cycle of the ancestral wolf, but the numbers are not sufficient to support such a conclusion. Suffice it to say that estrus *may* occur in any month of the year.

Among the F_1's, all first estrus periods occurred in the latter half of the year. This partly reflects the fact that all the F_1's born from basenji mothers were approximately six months old at the end of June. However, the combined records of five animals kept over a period of years show a marked excess of heat periods occurring in the latter part of the year, even if the first estrus periods are eliminated. Figure 3*B* also shows two peaks for estrus, in February and August, six months apart.

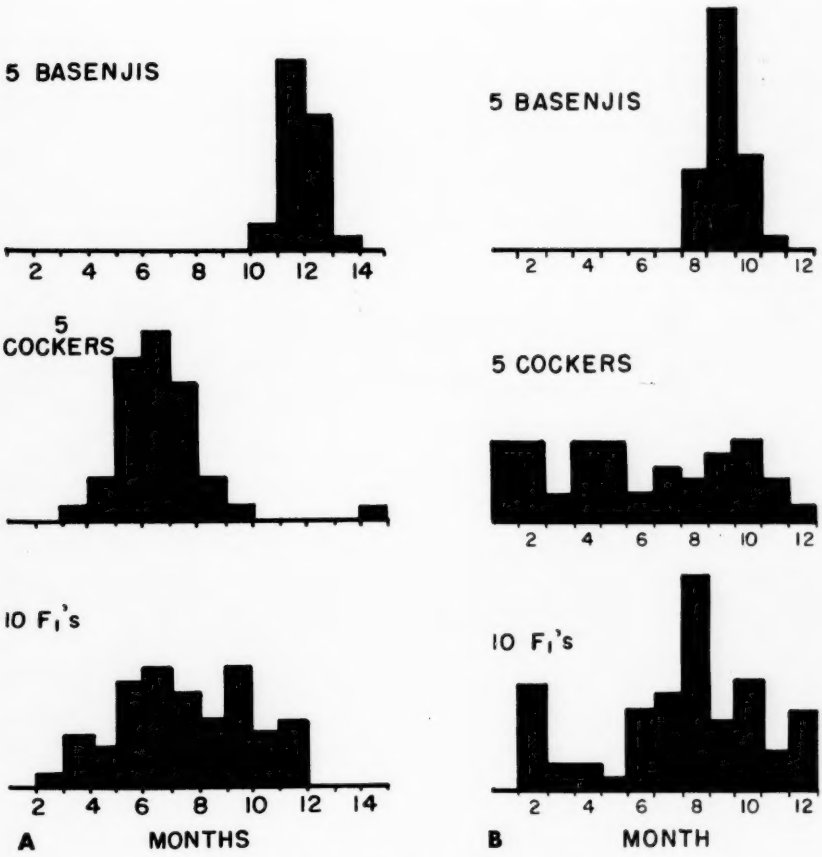
Let us now consider the interval between estrus periods (Figures 3*A* and 4*B*). In basenjis this interval falls between 10 and 14 months, with the peak occurring around 12 months. Ninety percent of the intervals observed in the sample of five females fell between 11 and 13 months.

In cocker spaniels, the interval between the first two periods ranged between four and 11 months, with small peaks at five and nine, and continuous variability between the extremes. The later successive intervals on the smaller group of animals showed considerably less variability, with 80 percent of the intervals falling between five and eight months, the median being slightly over six months. It is possible either that the first interval is more variable or that these data simply reflect variability contributed by a larger sample of individuals.

The F_1 hybrids at first showed two types of intervals, a long one of nine to 11 months and a short one of two to three months. However, the intervals between successive heats on a smaller number of animals varied continuously from three months through 11 months. The intervals, therefore, overlap with both cockers and basenjis.

The F_1's thus have a tendency to run spring and fall cycles but with a tendency for the spring cycle to be suppressed. There is also some tendency to run two cycles in the autumn, as if both basenji and cocker spaniel cycles were operating. The result is to produce a significantly greater amount of variability than in the parent cocker strain (Table II).

This being the case, it is impossible to clearly differentiate F_1 animals from



INTERVALS BETWEEN ESTRUS CYCLES

Figure 3

A—Intervals between successive estrus cycles in pure breeds and F_1's. Note the high degree of variability in the F_1's, overlapping both parent strains. However, individual animals can be distinguished from basenjis, as they never run successive 12-month cycles. *B*—Months in which bleeding began in females maintained for several years. The first 10 cycles are included in the cockers, and as many as observed in the basenji and F_1's (up to eight cycles). Note the partial tendency in the F_1's for cycles to begin in the latter half of the year, but more variably than the basenjis.

either the cocker spaniels or basenjis on the basis of the interval between the first two heat periods, although no F_1 hybrids were ever observed to run two or more successive long cycles, as in the basenjis. The F_1's may run six-month periods or less, and they also may run 11-month periods, although only in about one-seventh of the cases. The least amount of overlap occurs between the F_1's and parent basenjis, suggesting that the basenji condition is inherited as a recessive.

Classifying the intervals as in Table I,

we can make estimates of the goodness of fit to the alternate hypotheses of one- or two-factor inheritance, based on the number of animals showing the basenji-like interval of 11 to 14 months. Three such animals occurred in the F_1, but these all belonged to one litter in which early growth and development was seriously retarded by illness of the mother. These animals remained distinctly smaller than the rest throughout life. Otherwise, the results are consistent with the assumption that the basenji condition is inherited as a recessive, since no such animals appeared in the backcross to the cocker spaniel. In the backcross to the basenji, nine out of 19 animals showed the basenji-type cycle, as did three out of 32 in the F_2's. The goodness of fit to a one-factor hypothesis is almost perfect for the backcross, and comes out at $P = .085$ for the F_2, a nonsignificant deviation. In contrast, the fit for the two-factor hypothesis departs from expectation with a probability of .02 in the backcross and is almost perfect in the F_2. If we assume that one-seventh of the animals with intervals between 11 and 12 months are heterozygous, since the F_1 hybrids show approximately this amount of overlap, this reduces the backcross ratio by only one animal, leaving the probability for a one-factor hypothesis at .94, which is still nonsignificant. The F_2 ratio is not affected. We may therefore conclude that the data fit the assumption of single-factor inheritance better than they fit a two-factor hypothesis.

Discussion

There are three points of general interest in these results. One is the occurrence of a higher degree of variability in the F_1 animals than in the parent strains, a phenomenon which occurs fairly often in behavioral data[3]. In this case the result cannot be accounted for by the assumption of heterozygosity in the parent strains. In other cases it has been suggested that the F_1 hybrids were genetically located near a threshold, so that random environmental factors threw them into one or the other parental type. In the present case there appears to be a fairly clearcut mechanism; namely, two different physiological processes, one inherited from each parent and both operating in the F_1. Naturally this is a case in which variance analysis, based on the assumption of equal and additive factors affecting a single physiological process, will not apply.

Second, this is another case of an inherited trait in dogs which can be explained on the assumption of one or two genes. One would theoretically expect multiple-factor inheritance, and we can only suggest that selection operating on a behavioral trait is likely to operate chiefly through major genetic factors, since it would be difficult to distinguish a minor genetic effect on behavior amid plentiful variation produced by the environment. Even in the present case, which appears to be so largely

TABLE II. Mean and standard deviation of intervals between estrus in pure breeds and F_1 hybrids

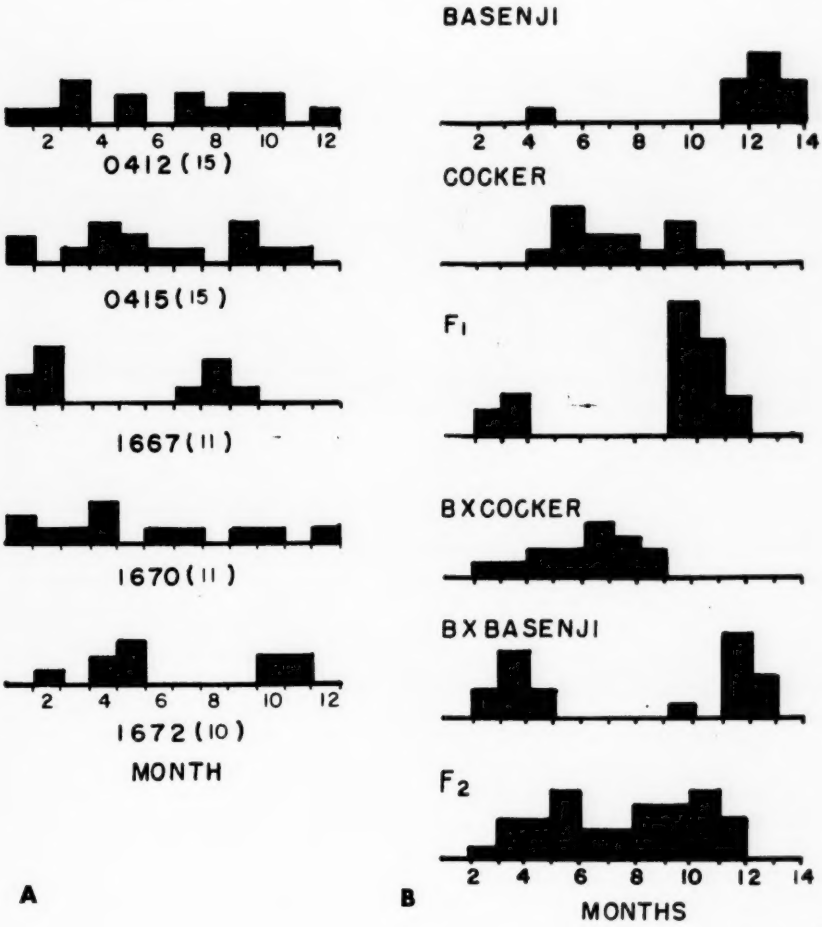
	Intervals between first two estrus periods	Intervals between successive estrus periods in groups of five animals
Basenji	12.50±0.77	11.87±0.69
Cocker	7.29±1.91	6.45±1.24*
F_1	8.74±3.60	7.50±2.41

*Aberrant 14 month interval omitted; probably result of heat period not observed.

TABLE I. Interval between first and second estrus periods

	Months				
Breed or Hybrid	0-4 (Typical of Heterozygotes)	4-9 (Typical of Cockers, Heterozygotes)	9-11 (Typical of Heterozygotes)	11-14 (Typical of Basenjis)	Total
Basenji	1*	0	0	11	12
Cocker Spaniel	1	10	3	0	14
F_1	5	0	17	3†	25
Backcross to Cocker	2	13	0	0	15
Backcross to Basenji	7	2	1	9	19
F_2	4	13	12	3	32

*Female ran second infertile cycle after losing pups
†All from same litter in which growth was retarded



VARIATION IN ESTRUS CYCLES

Figure 4

A—Month in which bleeding began in three cockers involved in the cross (lower), plus the mother (0415) and double grandmother (0412). Numbers in parenthesis indicate numbers of cycles observed in each animal. Note that some animals were highly variable, but some (1667 and 1672) ran fairly regular cycles at six-month intervals. *B*—Intervals between first two estrus periods, in months. Note indications of segregation in the backcross to the basenji, and wide variation in the F_1. F_1's can also show cycles of intermediate length, as shown in Figure 3*A*.

physiological rather than behavioral, there are indications that the basic cycles can be modified by environmental differences in nutrition.

Finally, there is the question of the evolution of this trait. According to the available evidence, the dog was probably domesticated from a subspecies of the wolf, *Canis lupus,* in the Mesolithic Period in Central Europe. From this point it probably spread rapidly all over the world, including the Southern Hemisphere.

F_1 HYBRIDS

Figure 5

F_1 hybrids from a cocker spaniel mother. Male on left, female on right.

The wolf typically reproduces in the spring of the year, with most pups born in May, conception occurring in March, and bleeding beginning in January or even late December. In the European breeds of dogs this has been modified into a six-month cycle with considerable variability, so that estrus may occur at any season during the year. The basenji is the only breed of dog known to depart from this cycle, but the Australian dingo shows in our latitudes a breeding cycle similar to the basenji, according to Dr. George Rabb of the Chicago Zoo. This suggests that early in the history of domestic dogs, those animals taken into equatorial regions developed a recessive mutation which shifted the cycle into the period of declining light rather than increasing light. This would also indicate a relationship between the basenji and dingo, which is supposed to have originated as a feral domestic dog.

We have no information regarding the effect of light changes upon the cycle of the wolf, nor about the breeding cycle of the basenji in its native equatorial Africa. An alternate suggestion to the above is that the wolf cycle is actually produced by declining light, since it begins soon after the first of the year, and that the genetic change in the basenji was one of increased sensitivity to declining light as an adaptation to tropical conditions. It would be interesting to try the effect of artificially simulating light conditions as they exist near the equator.

The chief imperfection in the data is the small number of subjects in certain populations. This is the unavoidable result of working with a character affecting only one sex. However, the numbers in two of the hybrids, the F_2's and backcross to the basenji, are sufficient to make any interpretation other than a single-factor recessive highly unlikely. The final crucial test for a single factor, that of repeated backcrosses, was not done, and we cannot completely eliminate the possibility of multiple factors.

The results also show how a relatively simple genetic mechanism may interact with environmental factors to produce a highly complex manifestation of a phenotype. The variable length of interval in the heterozygous animals is influenced in part by the season of birth, since all animals which ran their first two cycles in the fall of the year and hence showed an interval less than four months, were born in the period from August through January. Since the hybrid populations born to basenji mothers were necessarily born in this period, and other hybrids were born throughout the year, the result is the wide variation of intervals seen in Table I.

Summary

The African basenji typically exhibits an annual breeding season in the autumn of the year, in contrast to other dog breeds in which the females run cycles approximately six months apart at any season of the year.

The results are based on the time of onset of bleeding in females obtained from a Mendelian cross between basenjis and cocker spaniels.

The ratios obtained are consistent with the hypothesis that the basenji breeding cycle is produced by a single recessive gene, but the possibility of multiple-factor inheritance cannot definitely be excluded.

The heterozygotes exhibit a high degree of variability in the expression of the trait, with cycles partially concentrated in the last six months of the year, so that the intervals may be either longer or shorter than those typical of cockers. Hence the F_1 is more variable as a population than either parent strain.

The possible origin of the seasonal cycle in basenjis is discussed in relation to the history of domestic dogs.

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◆

Epistasis—What is it?

FOR some time we have been impressed with the confusion that exists regarding the terms "epistatic" or "epistasis." In Knight's *Dictionary of Genetics*[4] it is defined as "Dominance of one factor over another—the two not being allelic. Such a dominant factor is said to be epistatic and the non-allelomorphic recessive hypostatic." This seems perfectly straightforward. However, it is used in a variety of meanings by different authors. It is generally agreed among most authors that "epistasis" refers to two genes that are not allelic. In a recent book by Little[5], however, on inheritance of coat color in dogs he uses it to denote

degrees of dominance in a multiple allelic series, as follows:

"When more than two alleles have been identified at a single locus, they are called *multiple alleles*. In the dog four loci for coat color have been identified which may contain any one of four alleles, and six which contain two each.

"Whenever three or more alleles are found, they can usually be classified and arranged in the order in which they can mask, conceal, or cover the expression of the other members of the series. The word *epistatic*, 'placed above,' is applied to a gene that conceals one or more others, while the term *hypostatic* denotes 'standing under,' and is applied to the concealed member of the series. Thus in a series of three there will be a top-ranking member, epistatic to both the others, a middle member, hypostatic to the top member but epistatic to the other member, and the bottom member, which is hypostatic to both its partners.

"In the ordinary pair of Mendelian units the one of the pair which masks or conceals the other is called *dominant*, and the one which is masked, or concealed, *recessive*."

Little's use of the terms "epistasis" and "epistatic" is diametrically opposed to that of Knight. Both definitions have something in common, however. They agree that the gene or allele that acts as a "dominant" should be placed higher in the epistatic series than one which is masked or concealed.

It might be well to turn to the original use of the terms "epistatic" and "hypostatic." Actually these terms were introduced by Bateson in 1909[1], just a half century ago. During this half century the meaning has evolved in two rather divergent directions. It seems highly appropriate at this time to try to arrive at a single meaning, if that is possible.

Bateson introduced these terms in connection with coat color determiners in mice. Three different color determiners were being considered: agouti versus non-agouti, black versus chocolate, and colored versus albino. The last was due to a single gene which we now call *C/c*, *C-* being colored and *c/c* albino. Bateson properly used the term dominance to describe the masking effect of *C-*, over *c/c* the recessive. He states, "The term is thus used correctly to denote the relationship between allelomorphic features *belonging to the same pair*.* But confusion will be introduced if we extend the same term to the relationship between various determining factors which belong to distinct allelomorphic pairs.

"Hitherto we have spoken of the determiner for such color as grey in rabbits and mice as 'dominant' over the colors lower in the scale, such as black and chocolate. Nevertheless we are here dealing with a relationship quite different in order from that subsisting between the colored and the albino. *Pending a more precise knowledge of this relationship** it will be enough to regard those factors which prevent others from manifesting their effects as higher, and the concealed factors as lower. In accordance with this suggestion the terms *epistatic* and *hypostatic* may conveniently be introduced. We shall then speak of the determiner for grey as epistatic to that for black; that for black epistatic to that for chocolate, and so on."

From this definition by the introducer of the term, it is clear he intended that the factors which acted as dominants were called epistatic —pending a more precise knowledge of the relationship. For alleles of genes at the same locus he used the well-known term dominance, introduced by Mendel. According to Bateson's usage an allele might change from an epistatic one to a dominant one as soon as it was established that they were alleles. We are somewhat at a loss to understand Little's use of the terms epistatic and hypostatic for alleles at the same locus. In this instance dominance and recessive might well be used. The terms epistatic and hypostatic have gradually come to be used to indicate dominant and recessive relationships for genes not at the same locus, i.e., nonallelic, as Knight's definition states. For alleles, dominant and recessive serve admirably well.

Another source of confusion exists when interaction of genes is considered. To produce a normal wild-colored mouse requires the interaction of genes *A/-* for agouti, *B/-* for black, and *C/-* for color. In the presence of *c/c* no color develops. The animal is an albino. Since this *c/c* precludes the expression of genes *A* and *B* some authors prefer to label *c/c* as epistatic to both *A* and *B*. We must avoid confusing gene interaction and function with the inheritance of the same gene. If a gene behaves as a recessive, as does *c*, then it is a recessive, being allelic to *C*. If it were nonallelic we would call it hypostatic.

Dodson[2], in a sub-topic on "Hierarchies of Genes" discussing coat colors in mammals, lists the albino series at the top of the hierarchy. In the presence of *C/-* color develops, while in the presence of *c/c* an albino develops. Since these alleles permit or preclude the expression of any of the other colors, such as the black, agouti, brown, etc., Dodson refers to the *C/c* alleles as being epistatic to the others.

If we carry this analogy to *Drosophila* it could be stated that the gene for white eye, *w/w*, is epistatic to all of the various eye colors, vermilion, scarlet, brown, etc., because the dominant allele *w+* must be present to allow these colors to develop. If we confine ourselves to alleles at the white locus, there is a variety of colors ranging through white (*w*), ivory (w^i), apricot (w^a), eosin (w^e), to the wild *w+*. According to Little's definition the

*Italics mine. W. R. S.

w^+ would be epistatic to all others; w^e, for example, would be hypostatic to w^+ but epistatic to w, and the white allele w would be at the bottom of the list. According to Dodson the w would be epistatic to all colors because it blocks the appearance of any color, if we interpret Dodson correctly.

If we used this same reasoning in biochemical mutants in neurospora, almost any biochemical mutant would be epistatic to the normal alleles since it is essentially lethal unless the missing metabolite is supplied. In fact, any lethal could be classified as epistatic. To so classify them does not clarify them but only confuses the picture. It seems Bateson was truly prophetic in predicting that there might be confusion regarding the use of the term epistatic.

The greatest variety of terms involving epistasis occurs in Snyder and David's "The Principles of Heredity."[6] They have dominant epistasis, dominant and recessive, recessive, duplicate dominant, duplicate recessive, duplicate genes with interaction, all under the heading of epistasis. Does the addition of the term epistasis to these well-known interactions of different genes really clarify the operation of the various genes to produce their phenotypic effect?

We are of the opinion that we should follow the simple definition given in Knight and not try to cover every different genetic interaction by the term *epistasis*. An excellent example of epistasis, we believe, is that of the competition between two genes affecting sexual development and expression in maize. The gene *sk/sk* causes no silks to be developed on the ear, essentially a male type. The gene *ts/ts* causes silks to be developed in the tassels and elimination of the anthers. This is essentially a pistillate or female type with no pollen being developed. What happens in the double recessive *sk/sk, ts/ts*? Jones[3] found that the double recessive had silks in the tassel *and* silks in the ear. Even the secondary florets on the ear developed. So the gene *ts/ts* had a strong female influence. We think the gene *ts/ts* can properly be said to be epistatic to *sk/sk*.

Another good example of epistasis seems to be the dominant gene for gray, *G*, in horses. Animals with the *G/G* or *G/g* constitution become gray, some at an early age, completely masking the original color whether it be black, bay, or chestnut. Another example is the black gene in cattle, this color being dominant to the red of such breeds as the Hereford and Shorthorn, or the brown of the Jersey and Guernsey. These genes are nonallelic, and black could properly be called epistatic.

This may seem a trivial controversy, but what is a teacher going to teach about epistasis?

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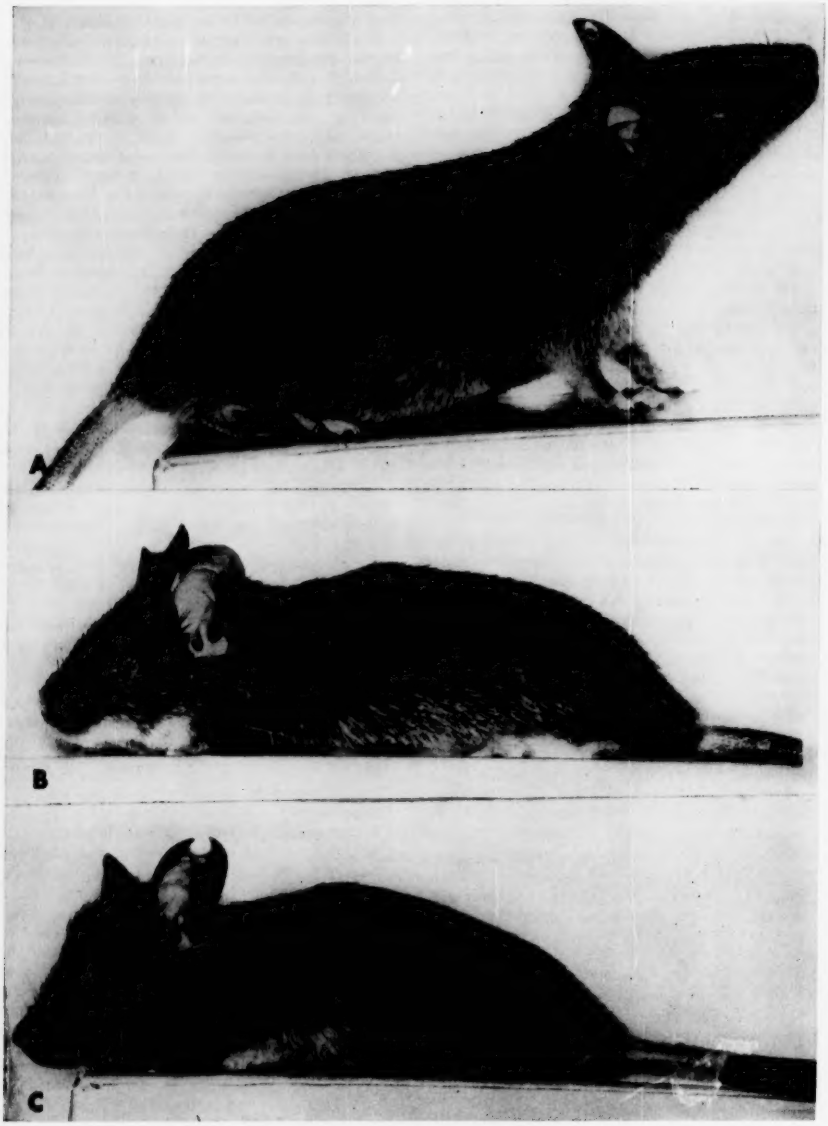


BRAIN HERNIA MUTATION

Figure 6

A—Normal adult mouse. *B*—Adult *bh/bh* mouse. The head is essentially normal except for absence of the eyes. *C*—A mouse with extreme brain hernia and unilateral anophthalmia.

BRAIN HERNIA, A NEW RECESSIVE MUTATION IN THE MOUSE

DOROTHEA BENNETT*

A NEW mutation, which is known so far to affect the development of the head and of the kidneys, appeared about a year ago in the second generation of an outcross between the Bar Harbor AKR-T stock (kindly supplied to this laboratory by G. D. Snell and E. S. Russell) and our own inbred Brachy stock. The most striking feature of many of the mutants first seen was a protrusion of part of the brain through a median dorsal opening in the skull; the mutation was therefore called "brain hernia" (symbol *bh*). It is not known whether the mutation had been present in either of the stocks prior to the outcross, but there has been no evidence of it in that part of our Brachy stock which is still maintained as an inbred line. The AKR-T stock was being bred at that time for the investigation of another apparent mutation, *minature* (the description of which will be published separately) which had appeared in the third generation of inbreeding in our laboratory.

Segregation Data

Brain hernia appears to behave as a simple Mendelian recessive (Table I). Animals were classified as $+/bh$ because of (1) normal phenotype, and (2) the production of a litter containing at least one offspring with the brain hernia phenotype. In subsequent *inter se* matings, these heterozygotes produced at birth a total of 351 normal: 108 with the brain hernia syndrome. No sex difference was found. This ratio does not differ significantly from the 3:1 ratio expected from matings between heterozygotes for a simple autosomal recessive with complete penetrance and expressivity, and normal viability in homozygotes at birth. Outcrosses between $+/bh$ animals and known $+/+$ mates have resulted only in normal offspring. Recently, a few homozygous bh/bh have proved fertile, and matings among them have produced 17 with the brain hernia phenotype, and one apparently normal animal, probably a rare normal overlap.

Linkage Data

Because of the primary interests of this laboratory, the possibility of linkage between *bh* and *T* was investigated. The test was made in the following way: one male, heterozygous for both *T* and *bh*, was bred to a large number of females which were $+/+$ at *T* locus (normal-tailed) and heterozygous for *bh*. If *T* and *bh* were relatively closely linked and thus necessarily either in coupling or repulsion in the single male used, we should expect his bh/bh progeny from such matings to be primarily either bh/bh Brachy or bh/bh normal-tailed. If the genes were on different chromosomes, independent assortment should be the rule. The results obtained were 15 normal-tailed bh/bh: 12 Brachy bh/bh.† It appears therefore that *T* and *bh* are not linked, or only very loosely so.

TABLE I. Segregation data

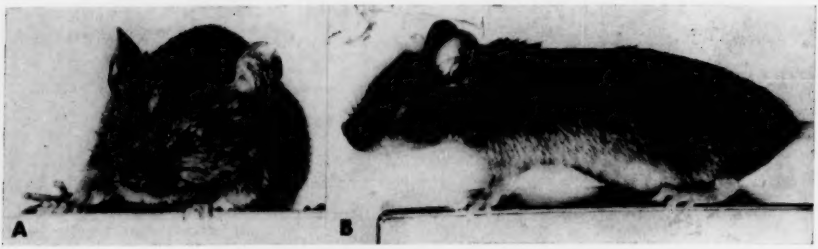
Mating	Offspring: Normal ($+/+$ or $+/bh$)	Offspring: bh/bh	total
$+/+ \times +/bh$	26	0	26
$+/bh \times +/bh$	351*	108	459
$bh/bh \times bh/bh$	1**	17	18

*19 of this group were progeny tested with known $+/bh$ mates; they proved to be 12 $+/bh$: 7 $+/+$, i.e. not significantly different from the expected 2:1 ratio.

**Presumably a normal overlap.

* Department of Zoology, Columbia University, New York 27, N. Y. Supported in part by Grant NSF-G-3124, from the National Science Foundation.

†We are grateful to Mr. Bill Boland for collecting part of this data.



EXTREME BRAIN HERNIA TYPES

Figure 7

A—This is another view of the mouse shown in Figure 6*C*. *B*—Shows an extreme hydrocephalic type, with apparently normal eyes.

Description

At birth *bh/bh* animals show externally a rather wide range of head abnormalities. The most striking is cerebral hernia which may vary from a barely noticeable blister to a bloody sac several millimeters across. The herniated brain is always covered with skin, and, surprisingly, is only very rarely broken by the birth process. The condition of herniated brain is often but not always accompanied by some degree of hydrocephaly. Many *bh/bh* newborns show no frank brain hernia, but only varying degrees of hydrocephaly. In addition, most animals seem to have foreshortened heads. It has been interesting to note that the most extreme manifestations of herniated brain are not usually accompanied by hydrocephaly, and conversely that marked hydrocephaly does not occur with cerebral hernia. As well as these defects of the brain and skull, approximately 75 percent of *bh/bh* newborns are obviously microphthalmic or anophthalmic. Table II gives the distribution of these anomalies in a group of animals examined externally shortly after birth. It appears that eye defects are somewhat more likely to occur with cerebral hernia than with simple hydrocephaly. The eyes are not always affected in a symmetrical fashion; the figures in Table II indicate that when there is dissymmetry there is a marked tendency for the eye defect to occur on the left. In some adult animals with eyes of apparently normal size opacity of the lens has been noted. No other externally visible abnormalities have been observed.

Eight newborn *bh/bh* animals were prepared as cleared whole mounts stained with alizarin red. A study of these has revealed that in both cerebral hernia and hydrocephalic types, the most extreme skull defect is a wide median separation of the paired frontal and parietal bones. In less severely affected animals the parietals may meet in the dorsal midline, but the frontals are always separated along at least part of their length. An interfrontal was present in all cases. In all skulls studied, the orbits were of normal size, regardless of whether the eye

TABLE II. Distribution of head abnormalities in animals scored at or shortly after birth

			% with eye defects
Brain hernia only	11	9/11	82
Hydrocephalus only	38	27/38	71
Brain hernia & hydrocephalus	14	13/14	92
Eye abnormalities only	2		100

TABLE III. Distribution of eye defects in animals scored at or shortly after birth

Condition		No. of animals
Anophthalmia	Symmetrical	3
	Left only	4
	Right only	1
Microphthalmia	Symmetrical	26
	Left side only or more severely affected	15
	Right side only or more severely affected	2
Apparently normal		8

EXTREME CEREBRAL HERNIA
Figure 8
A and *B*—Two views of newborn mouse with extreme cerebral hernia and reduced eyes (left) and normal littermate.

KIDNEY SECTIONS
Figure 9
A—Section of kidney of normal adult mouse. *B* and *C*—Sections of kidney of two adult *bh/bh* mice showing cystic condition.

it contained was reduced in size or absent.

As pointed out above, the viability of *bh/bh* homozygotes appears to be normal during intra-uterine life and the immediate postnatal period. In later life, however, their viability is definitely subnormal, although the increased mortality does not seem to have any positive correlation with the severity of the head defects. Of 40 animals classified as *bh/bh* at birth, only 18 were still alive four weeks later, and some of these were small and sickly. This rate of attrition is much greater than the usual mortality between birth and weaning at four weeks which in this laboratory is about 10 percent. The fertility of *bh/bh* animals surviving into adulthood has not been measured in any systematic way, but our impression is that some of them are almost certainly sterile, while others breed very well.

In the course of routine autopsies of five *bh/bh* adults it was noted that the outer surface of the kidneys was always irregular and nodular. Histological examination of the kidneys from two adult *bh/bh* animals revealed a polycystic condition, which was confined to the cortex, and characterized by the dilation of many of the glomerular spaces, and tubules. The ureters were not noticeably enlarged. Gross inspection of the kidneys of 20 externally normal (+/+ or +/*bh*)

adult mice of the same strain did not give any evidence of abnormalities. Polycystic disease appears therefore to be regularly associated in adults with the *bh/bh* genotype. Careful histological study of the kidneys of two newborn *bh/bh* and two normal littermates did not, however, reveal any polycystic tendencies in the mutants. Gross examination of 22 additional *bh/bh* newborns was also negative. It seems then, that polycystic disease must develop in *bh/bh* animals sometime between birth and four or five weeks. No other visceral abnormalities were detected in mutant animals.

Comparison with Other Mutants

Numerous other mutations in the mouse are already known which influence one or more of the same elements affected by *bh*. *Ur* (urogenital), described by Dunn and Glucksohn-Schoenheimer[4]; produces in homozygotes foreshortened head and cleft palate as well as kidney abnormalities involving in part inflation of Bowman's capsule which appears during post-natal life. (Other kidney abnormalities present in *ur* but not in *bh* include absence of one or both kidneys, considerable tubular dilation, and hydro-ureter.) Another gene or gene complex *my* (mylencephalic blebs) which was discovered in a stock X-rayed by Little and Bagg[6] produces abnormalities of eyes, feet, and kidneys. Bagg later was able to select a stock in which abnormalities were largely confined to the eyes and kidneys[1]. The eye abnormalities included frequent extreme microphthalmia, as well as some defects of the eyelids not seen in *bh*, while the kidney abnormalities include polycystic disease, but also hydroureter and complete or partial absence. In spite of the obviously rather wide differences among these various mutants, there seems to be some evidence for a possible developmental or physiological relationship between abnormalities of the eyes and head, and the kidneys.

One other genetically determined condition involving herniated brain has been described. This is *st* (shaker-short), found by Dunn[3] and described in some detail by Bonnevie[2]. In this case the cerebral hernia appears at the occipito-parietal junction, but otherwise seems similar to the one described in this paper. Associated with *st* are waltzing behavior and a short tail; to our knowledge the adult kidney has not been examined.*

Several other mutations producing hydrocephaly also exist in the mouse: *ch*, *hy*-1, *hy*-2, *hy*-3. (Review in Grüneberg)[6]. Apparently neither cerebral hernia nor kidney abnormalities are concomitants of any of these conditions.

Summary

A new recessive mutation *bh* (brain hernia) has arisen in the descendants of an outcross between the Bar Harbor AKR-T stock, and the Columbia Brachy stock. At birth homozygotes have one or more of the following conditions in varying degree: cerebral hernia, hydrocephaly, anophthalmia, microphthalmia. Later in life, apparently all of them develop polycystic kidneys.

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* No behavioral disturbances have been seen in *bh/bh*, regardless of the extent of deformation of the brain and skull.

INHERITANCE OF WHITE COAT COLOR IN THE WATER BUFFALO OF THAILAND

DAVID C. RIFE AND PRASOB BURANAMANAS*

WATER BUFFALO OF THAILAND

Figure 10

Gray and white water buffalo in a field near Chiang Rai, Thailand.

THERE are between five and six million head of water buffalo throughout Thailand, reared almost exclusively for draft purposes. They are of the same variety as those used in the Philippines, and are sometimes called *carabao*. The majority of these water buffalo have dark gray body color and white stockings. About 10 percent of the water buffalo in Thailand, however, have white hair over the entire body, overlying pink skin with brown spots. Gray water buffalo have black skin. Both gray and white water buffalo have dark eyes.

The incidence of white color varies considerably in different regions of the country, ranging from about 30 percent in the extreme north to less than 5 percent in southern regions. White water buffalo are rumored to be less vigorous than the gray, and bring slightly lower prices than the gray. But there is no evidence that there has been any effective and consistent selection for gray in buffalo breeding. It is common practice to graze all the water buffalo in a community in a single herd, often containing both gray and white bulls. It appears that mating occurs on a random basis, at least as far as coat color is concerned.

Interviews with numerous farmers concerning the results of different matings with respect to coat color were unsatisfactory from the standpoint of obtaining accurate information. The only source of completely accurate information regarding the inheritance of white color proved to be observations of the frequencies of coat color combinations among dams and their suckling calves. If coat color is simply inherited, it should be possible to learn how, on the basis of

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the following types of information within each region from which samples are taken.

1. The percentage of white calves from gray dams.
2. The percentage of white calves from white dams.
3. The percentage of gray calves from gray dams.
4. The percentage of gray calves from white dams.

When the foregoing data are assembled we may then test for dominance of gray or white. Let D represent the dominant gene, and R the recessive gene.

Genotypes	Phenotypes
DD	Dominant
DR	Dominant
RR	Recessive

Then let p = frequency of D within a population.

q = frequency of R within a population.

Gene frequencies $= p + q = 1$

Genotypic frequencies $= (p + q)^2 = 1$

If there is no selective mating for color, or if there is no difference in survival values of either gray or white, the expected proportions of various dam-calf color combinations may be easily calculated, as follows:

Let q = frequency of recessive gene (R).

As q^2 = frequency of recessive phenotype in the buffalo population, q^3 = proportion of R dam — R calf combinations.

Therefore $q^2 - q^3$ = proportion of R dam — D calf combinations.

TABLE I. Tests for dominance of white

Sample 1

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$	
GG	242	236.8	5.2	0.09	
GW + WG	21	31.8	10.8	3.66	($q = .940$)
WW	22	16.4	5.6	1.91	
Total	285	285		$\chi^2 = 5.66$ d.f. $= 2$	

Sample 2

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$	
GG	97	101.7	4.7	0.21	
GW + WG	38	28.5	9.5	3.51	($q = .877$)
WW	15	19.8	4.8	1.16	
Total	150	150		$\chi^2 = 4.88$ d.f. $= 2$	

Sample 3

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$	
GG	483	487.1	4.1	0.03	
GW + WG	110	100.6	9.4	0.87	($q = .907$)
WW	60	65.3	5.3	0.04	
Total	653	653		$\chi^2 = 0.94$ d.f. $= 2$	

Total of All Samples

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$
GG	822	825.6	3.6	0.01
GW + WG	169	160.9	8.1	0.40
WW	97	101.5	4.5	0.19
Total	1088	1088		$\chi^2 = 0.60$ d.f. $= 2$

TABLE II. Tests for dominance of gray

Sample 1

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$	
GG	242	230.6	11.4	0.56	
GW + WG	21	43.2	22.2	11.40	($q = .339$)
WW	22	11.2	10.8	10.80	
Total	285	285		$\chi^2 = 22.76^{**}$ d.f. $= 2$	

Sample 2

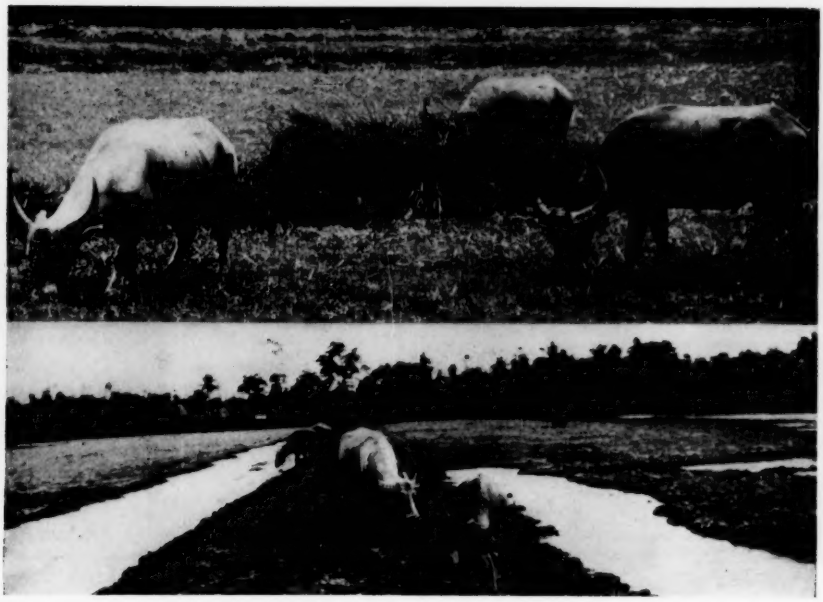
Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$	
GG	97	98.1	1.1	0.01	
GW + WG	38	35.7	2.3	0.15	($q = .476$)
WW	15	16.2	1.2	0.08	
Total	150	150		$\chi^2 = 0.24$ d.f. $= 2$	

Sample 3

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$	
GG	483	470.1	12.9	0.35	
GW + WG	110	134.6	24.6	4.52	($q = .420$)
WW	60	48.3	11.7	2.83	
Total	653	653		$\chi^2 = 7.70^{*}$	

Total of All Samples

Combinations	O	C	O − C	$\frac{(O-C)^2}{C}$
GG	822	798.8	23.2	0.67
GW + WG	169	213.5	44.5	9.23
WW	97	75.7	21.3	5.99
Total	1088	1088		$\chi^2 = 15.89^{**}$



COAT COLORS OF WATER BUFFALO

Figure 11

Gray and white water buffalo in a field near Kasetsart University, Bangkok, Thailand, (above), and Paknam, Thailand, (below).

If the population is in equilibrium and there is no selection, the proportion of R dam — D calf combinations should be the same as D dam — R calf combinations.

Therefore $q^2 - q^3$ = proportion of D dam — R calf combinations.

The total proportion of dam-calf combinations unlike in color $= 2(q^2 - q^3)$. Summarizing:

q^3 = expected R dam — R calf combinations.
$q^2 - q^3$ = expected R dam — D calf combinations.
$q^2 - q^3$ = expected D dam — R calf combinations.
$1 - (2q^2 - q^3)$ = expected D dam — D calf combinations.

Actual Data

Three samples of dam-calf data were obtained; 285 pairs from regions near Bangkok, 150 pairs near a road in northeastern Thailand, and 653 pairs from a northeastern region removed from the highway (the latter sample was obtained by the castration unit in the region). These samples shall henceforth be referred to as samples 1, 2, and 3 in the order listed above. They total 1,088 pairs. Somewhat different gene frequencies were noted, the highest frequency of white being in sample 2, the lowest frequency in sample 1.

Gene frequencies were obtained from the proportions of gray and white animals in the entire sample of dams and calves. In tables symbols shall be used to designate the various dam-calf combinations as shown below:

GG — Gray dam, gray calf
GW — Gray dam, white calf
WG — White dam, gray calf
WW — White dam, white calf
q — Frequency of recessive gene
d.f. — Degrees of freedom
* — Significant deviation
** — Highly significant deviation
O — Observed frequency
C — Calculated frequency

The tests agree closely with the hypothesis that white is dominant to gray, and none of the samples show significant differences between observed and calculated frequencies. The tests do not support the hypothesis that gray is dominant to white. Sample 1 shows a highly significant deviation, and sample 3 a deviation from the expected. The total shows a tremendous deviation from the expected.

The data, therefore, strongly support the hypothesis that white is dominant to gray color in the water buffalo of Thailand.

TABLE III. Proportion of gray dam, white calf to white dam, gray calf

Sample	GW	WG	Total
1	14	7	21
2	24	14	38
3	65	45	110
Chiang Rai	19	9	28
Total	122	75	197

	O	C	O − C	$(O-C)^2$ / C
WG	122	98.5	23.5	5.61
GW	75	98.5	23.5	5.61
Total	197	197		$\chi^2 = 10.32^{**}$

Proportions of GW to WG

Unless there is selection of some sort, WG and GW should be expected to occur in approximately equal numbers. Yet all three samples, plus additional observations made near Chiang Rai, show consistently greater numbers of GW than WG. This is summarized in Table III.

The differences between GW and WG are consistent and very highly significant. Some sort of selection appears to be operating in favor of white calves, as there is an increase of around four percent in the proportion of white calves, as compared with white dams.

Sex-linkage and sex-limited inheritance cannot account for the increase in white, as the two colors appear to occur in both sexes in approximately the same frequencies.

Summary

Data obtained from a survey of 1,088 pairs of dams-calves of water buffalo in Thailand strongly suggest that white is dominant to gray coat color.

The proportion of white among calves is approximately four percent greater than among their dams. The reason for this increase is as yet unknown.

CHROMOSOME ELIMINATION IN A TRISOMIC OF SPINACIA OLERACEA

J. R. Ellis and Jules Janick*

THE six primary trisomics of *Spinacia oleracea* ($n = 6$), named in accordance with differentiating vegetative characters[2], have been associated with their respective chromosomes[1]. Plants trisomic for chromosome 4 (*curled*) have shown instability with respect to chromosome number. The *curled* trisomics are characterized by stunting and by rigid leaves that are markedly twisted, as shown in Figure 12*A*. In a recent study five *curled* trisomics were initially selected from progeny of triploid pistillate × diploid staminate crosses on the basis of chromosome counts in root tips. Two of these *curled* trisomics subsequently ceased growing due to a necrosis of the apical meristem. All of the

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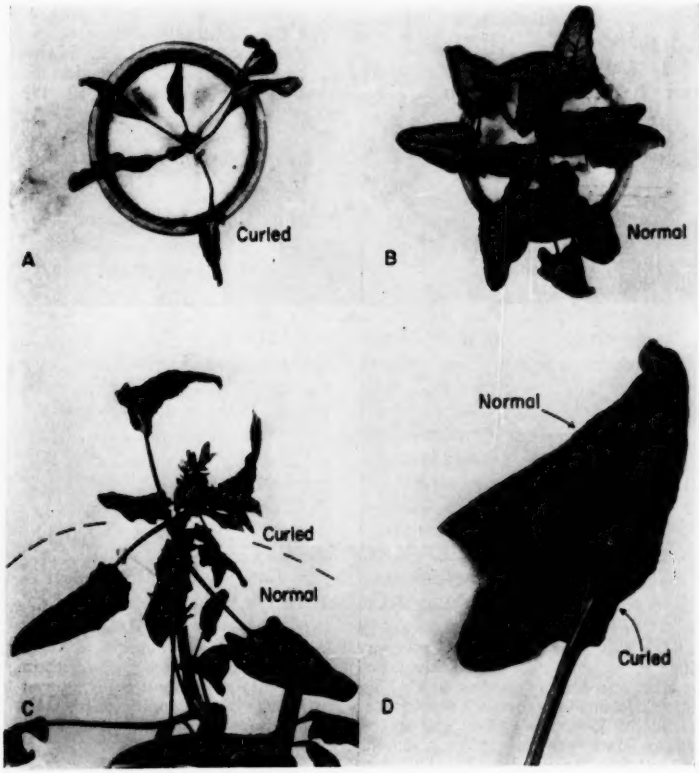

CURLED TRISOMIC AND NORMAL DIPLOID OF SPINACH

Figure 12

A—*curled* trisomic; *B*—normal diploid plant; *C*—chimeral plant, half normal and half *curled*; *D*—sectored leaf, half normal and half *curled*.

other three plants have shown a reduction in chromosome number in the root tips from 13 to 12 during a period of four months. When this phenomenon was first observed, all of the root tips sampled from two of the plants had 12 chromosomes. However, out of 10 roots sampled in the third plant, only one root had 12 chromosomes, the other nine roots having the trisomic complement of 13 chromosomes. The chromosome eliminated was that which initially was present in the trisomic condition.

These three plants were induced to form seed stalks with an 18-hour photoperiod. The aerial portion of two of these plants remained unaffected—i.e., retained the trisomic phenotype. However, one of the plants produced a *curled* phenotype in only half of the plant, the other half being normal. The chimeric nature of this plant is shown in Figure 12*C*. One leaf of the plant was sectored along the midvein, with one-half of the leaf *curled* and the other half normal as shown in Figure 12*D*.

This phenomenon of chromosome elimination has resulted in a sectoral chimera involving both roots and shoots. While chimeras involving genomes or particular gene mutations are well documented, this is believed to be one of the few cases

produced as a result of a single chromosome difference.

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TELEGONY RETESTED

Joseph C. Daniel, Jr.*

TELEGONY is the belief that previous matings of a female will genetically influence her later offspring by another male. This belief enjoyed common acceptance among animal breeders of 30 or more years ago and is still held by some today, in spite of several organized attempts to disprove it. The persistence of this belief, viewed in the light of more modern findings on the transmission of DNA (desoxyribose nucleic acid) made a retesting of the idea of telegony seem desirable.

DNA is generally accepted to be the basic structural material of the gene. As such, it is transmitted via the gametes, from parent to offspring. Recent work shows that it can be transmitted from one individual to another, in other ways. Some years ago, O. T. Avery and his co-workers[1] initiated studies on different strains of bacteria that have shown that DNA from one type can be incorporated into the genetic constitution of another type by being added to the growth medium. More recently, Benoit and others[2] succeeded in transferring characteristics from khaki ducks to white ducks, by injecting DNA from the former into the latter. Investigations[4] started in 1952 indicate that genetic characters (presumably DNA based) can be transduced by phage transmission from one bacterium to another. If DNA can be transmitted by these methods, it might also be transmitted through other routes that are natural to living systems.

During coition, millions of DNA-containing spermatozoa are deposited in the body of the female and those not used in fertilization are presumably absorbed by her. If this foreign DNA could influence her gametes, then her later offspring might be expected to show this influence in their genetic constitution, and thereby furnish a basis for telegony.

This hypothesis was tested by a series of controlled breeding experiments using genetic strains of rats and fruit flies.

Twelve albino rats (six of Sprague-Dawley and six of Wistar strains) were bred to genetically pigmented males. Two strains of males were used, Canadian hooded (black and white piebald) from the University of Wisconsin colony and black agouti Irish from the University of Miami colony. A third strain of pink-eye agouti piebald rats was tried but later discarded because of impotency. The females were permitted to have from one to three litters by the pigmented males, after which they were bred to albino males of their same strain and the offspring checked for any phenotypic alteration. Coat color, eye color,

(*Continued on page 298*)

*Adams State College of Colorado, Alamosa. The author is indebted to Dr. W. F. Dunning and Mr. William Strauss for furnishing the strains of rats and Dr. James Craft for help in proofreading and organization.

EVIDENCE OF GENETIC CONTROL OF BLOOD POTASSIUM CONCENTRATION IN SHEEP

J. F. KIDWELL, V. R. BOHMAN, M. A. WADE, L. H. HAVERLAND AND J. E. HUNTER*

EVANS[2] and Evans and King[3] studied the concentration of sodium and potassium in the red blood cells of sheep of the Southdown, Suffolk and Scottish blackface breeds. They observed: (1) two mutually exclusive types with respect to potassium concentration, high potassium (HK) and low potassium (LK), (2) sodium concentration varies inversely with potassium concentration, (3) the trait appeared to be inherited on a simple Mendelian basis, LK being dominant and HK recessive, (4) the proportion of the two types varied among breeds. Kerr[4] examined sodium and potassium concentration in the erythrocytes of the oriental fat-tailed sheep. The individuals were arbitrarily grouped into three classes, low potassium, high sodium; high potassium, low sodium; high potassium, high sodium. Abderhalden[1] observed concentrations similar to those of the first mentioned group.

This paper presents an analysis of similar data obtained from representatives of the Rambouillet, Hampshire, Columbia and Suffolk breeds as well as Rambouillet × Columbia and Rambouillet × Suffolk crossbreds.

Experimental Animals

A total of 311 sheep distributed among four breeds and two crossbred groups from flocks on two University of Nevada experimental farms were involved in this investigation. Table I indicates the distribution by breed and location and the mean potassium concentration. Since analysis of variance indicated no differences due to sex or age within a breed these classifications have been omitted. Sheep maintained on the Valley Road Farm have been used primarily for internal parasite control studies for many years and the animals involved in this study carry exceptionally heavy parasite infestations. Hematocrit and hemoglobin concentration is, consequently, much lower than normal. Sheep on the Main Station Farm appear to carry about normal parasite loads.

The Valley Road Flock has been maintained continuously with only occasional introduction of a ram since about 1910. The Main Station Farm Suffolk flock was established in 1955. The Main Station Rambouillet flock consists of three small inbred lines established in 1955 and a flock of aged range ewes and subsequent ewe lambs obtained in 1956. All except the range ewes are "purebred."

Blood samples were drawn by jugular venepuncture into tubes containing ammonium oxalate. Preliminary tests indi-

TABLE I. Distribution of experimental animals by breed, location and mean potassium concentration, mg. percent

Breed	Valley Road Farm				Main Station Farm			
	LK		HK		LK		HK	
	Number	Average	Number	Average	Number	Average	Number	Average
Columbia	22	32.1	4	131.4				
Hampshire	74	38.0	7	144.5				
Rambouillet	78	26.8	1	123.0	66	26.1		
Suffolk					22	38.0	14	152
Ramb. × Columbia	5	27.9						
Ramb. × Suffolk					17	30.4	1	136

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cated no differences in the determination of sodium and potassium by the method employed when ammonium oxalate or heparin was used as the anticoagulant. Whole blood was diluted 1:100 with distilled water. This solution was analyzed for potassium and sodium with a Beckman Model DU flame spectrophotometer with a photomultiplier attachment. The wave lengths were 766.5 and 589.3 milimicrons for potassium and sodium respectively. Potassium was determined for all samples, but sodium, hemotocrit and hemoglobin was determined on a smaller stratified subsample.

Analysis of variance, regression and correlation analyses and binomial probability as described by Snedecor[5] were applied to the data as appropriate.

Results and Discussion

Potassium analysis indeed indicated two distinct groups. The low group varied from 17 to 52 mg. percent K and the high group from 106 to 187 mg. percent K. There were no intermediate or overlapping values. Several animals were reexamined after an interval of several weeks. The analysis agreed within limits of measurement error. In any event there was no change from high to low or vice versa. This is in agreement with the findings of Evans and King[3].

Effect of Hemoglobin ond Hematocrit

Since most of the blood potassium is in the erythrocytes the hypothesis that the differences in potassium concentration are a function of erythrocyte concentration was tested. A random subsample of 14 HK and 14 LK animals was selected and hematocrit determined. There was no difference in mean hematocrit of the two groups. In a much larger group (94) of LK individuals the regression of potassium concentration on both hematocrit and hemoglobin was estimated. In both cases the regression coefficient did not differ from zero, i.e., there was no regression. It appears reasonable to conclude, therefore, that the observed variation in potassium concentration is not a function of hematocrit or hemoglobin levels.

TABLE II. Mean sodium and potassium concentration in whole blood and plasma of high and low potassium sheep expressed as mg. percent

	High Potassium		Low Potassium	
	Sodium	Potassium	Sodium	Potassium
Whole blood	243.6	152.0	278.2	39.2
Plasma	326.8	39.8	334.1	27.4

Relations Between Sodium and Potassium in Whole Blood and Plasma

Sodium potassium relations were studied between and within HK and LK groups in plasma and whole blood, utilizing the subsample of 14 HK and 14 LK animals, (Table II). A *2* × *2* factorial analysis, taking account of the two potassium classifications and whole blood and plasma, was made. A significant ion level × blood fraction interaction was found for both sodium and potassium. Meaningful comparisons, therefore, must be made separately for whole blood and plasma. Whole blood sodium concentrations are significantly higher in the low potassium group than in the high potassium group, which suggests an inverse relation between sodium and potassium, as reported by Evans[2]. Detailed examination did not reveal the distinct separation observed for potassium, but a continuous range with much overlapping between groups. Within group regression analysis of sodium on potassium concentration indicated no relation between the two. It appears that although there is a real difference in blood sodium concentration between the two potassium level groups, there is no biometric relation between sodium and potassium concentration within a given potassium level.

Plasma potassium levels are lower than whole blood levels, but in the same order and the observed difference in plasma potassium concentration is real but smaller than for whole blood. No difference in plasma sodium concentration between the two groups was observed.

TABLE III. Breed differences in whole blood potassium concentration among low group

Breed	Mean, mg.%	—Rambouillet	—R × C	—R × S	—Columbia
Hampshire	38.0	11.5	10.1	7.6	5.9
Suffolk	38.0	11.5	10.1	7.6	5.9
Columbia	32.1	5.6	4.2	---	---
Rambouillet × Suffolk	30.4	3.9	---	1.7	
Rambouillet × Columbia	27.9	---	2.5		
Rambouillet	26.5	1.4			

D = 3.5. Differences above the dotted line are significant.

Breed Effects

Examination of Table I suggests considerable variation among breeds in mean potassium concentration. Differences among the high group were not significant. It is possible the limited numbers were not sufficient to detect small differences if they did exist. It was, in fact, estimated that it would require groups of about 30 animals to detect differences of the magnitude observed.

Real differences were found among the low group. Since there was no difference between the Rambouillets on the Main Station and Valley Road farms, they were treated as a single group. Differences were determined by the method of Tukey[6] as described by Snedecor[5]. These are summarized in Table III. There is no difference between the two blackface breeds, which are highest among the low group. They differ from the Columbias and Rambouillets and the latter two also differ. The crossbreds are intermediate.

Inheritance

The numbers of progeny resulting from the various matings are summarized in Table IV. Clearly these data are entirely too meagre to warrant any conclusions. Although they do not contradict the hypothesis of Evans and King[3] that high potassium is inherited as a simple recessive, they are insufficient to lend strong support. A number of matings of high × high and high × low animals known to have produced high offspring are required for critical tests.

The observed breed differences among the low potassium group and possible differences among the high group presents a further complication. It appears unlikely that these differences are environmental in origin. The two Rambouillet samples were from extremely divergent environments, yet the means were identical. This is of particular interest in view of the significantly different hematocrit levels in the two groups. On the other hand, one group of Rambouillets, the Hampshire and Columbia flocks have different mean potassium concentrations but have been maintained in a common environment for many years. The hypothesis of a single major gene and several modifying genes with different frequencies in the various breeds might be invoked, but in these data there is scant evidence for critical evaluation.

If it is assumed that high potassium is inherited as a simple Mendelian recessive, and that panmixia prevails with respect to this gene, it is possible to obtain point and interval estimates of its frequency in the various breeds sampled. These are presented in Table V. The Rambouillets clearly differ from the Columbias and Suffolks and probably from the

TABLE IV. Potassium phenotypes and numbers of progeny from matings of rams and ewes of several phenotypes

			Ewes					
			LK		HK		Unknown	
	Rams		Progeny					
Breed	Ident.	Type	LK	HK	LK	HK	LK	HK
Rambouillet	1	LK	5	0	0	0	2	0
Rambouillet	2	Unknown	7	0	0	0	3	0
Rambouillet	3	Unknown	4	0	0	0	3	0
Rambouillet	4	LK	12	1	0	0	3	0
Rambouillet	5	LK	13	0	0	0	3	0
Suffolk	6	LK	6	1	0	0	2	0
Suffolk	7	LK	7	1	1	2	0	0
Suffolk	8	LK	2	0	0	0	0	0
Suffolk	9	Unknown	2	2	1	1	3	2
Columbia	10	LK	13	1	0	2	1	0
Hampshire	11	LK	27	3	2	1	4	0

TABLE V. Percent of observed high potassium individuals with 95 percent confidence interval and estimated frequency of high potassium gene and 95 percent confidence interval assuming a single gene and panmixia

	Percent High Potassium Individuals		Frequency of HK Gene, %	
Breed	Point estimate	95% Confidence in	Point estimate	95% Confidence interval
Columbia	15.4	4.8 - 36.2	39.2	21.9 - 60.2
Hampshire	8.6	4.1 - 18.9	29.3	20.2 - 43.5
Rambouillet	0.7	0 - 4.3	8.4	0 - 20.7
Suffolk	38.9	24.4 - 59.1	62.4	49.4 - 76.9

Hampshires, and the Suffolks differ from the Hampshires while there may be no difference between the Columbias and Hampshires or between the Columbias and Suffolks.

The deficiencies of these data are clearly recognized. They exist because most of the sheep are used in a breeding experiment, hence matings designed specifically to provide data required for conclusive analysis are not feasible. These observations are published with the thought that other investigators may wish to extend them. It would also be of interest to investigate blood sodium and potassium levels in other species.

Summary and Conclusions

Blood potassium concentration was determined on 311 sheep representing four breeds and two crossbred groups from two physical locations. Blood sodium and plasma potassium and sodium were determined on smaller subsamples. On the basis of these data the following conclusions appear warranted:

Sheep may be divided into two mutually exclusive groups with respect to blood potassium concentration.

These differences are not a function of hematocrit or hemoglobin concentration.

There are no differences due to sex, age or location within breeds.

Sodium concentration of the high potassium group is significantly lower than in the low potassium group. Within groups, however, there is no relation between sodium and potassium concentration.

There are no obvious groupings on the basis of sodium concentration.

Plasma potassium levels are lower but similar to whole blood levels.

Among the low potassium group there are distinct differences in mean blood potassium concentration among breeds. These are more likely to be genetic than environmental in origin.

The exact mode of inheritance is not clear and the data do not warrant conclusions. The data do not contradict the suggestion of Evans and King of a single Mendelian gene. The differences in means among the breeds must be considered. One possibility is the existence of modifying genes, but there are no data to evaluate this suggestion.

On the assumption of a single gene and panmixia, gene frequencies are estimated and found to differ among breeds.

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A LINKED GENE DETERMINING THE LETHALITY USUALLY ACCOMPANYING A HEREDITARY FLUID IMBALANCE IN THE MEXICAN AXOLOTL

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IN an earlier report on this hereditary fluid imbalance in the Mexican axolotl (*Siredon*—or *Ambystoma—mexicanum*), it was classed by the writer[1] as a lethal character inherited as a simple Mendelian recessive. Its gene was designated by *f*. Continued study of this trait over several years now permits a more complete and accurate interpretation of its true nature.

The imbalance syndrome in the axolotl embryo, as originally encountered and reported, consisted in the accumulation of excess fluid, producing a distention of the head, suprabranchial region, and trunk, more or less in that order, with the fluid in the suprabranchial region being of somewhat variable occurrence. The affected embryos became recognizable at stages 24 to 27 of Harrison's series, although the accumulation of excess fluid doubtless began at somewhat earlier stages. Although the gills developed at the expected time they soon became abnormal, with a reduced number of filaments, and remained subnormal in size even when a normal gill circulation had become established. This characteristic feature was considered to be due merely to damage incident to the earlier distention of the head and suprabranchial region. The affected larvae usually hatched but ordinarily never fed; only two out of over 2,100 were known to have begun to eat, and neither survived to maturity.

Some time after the above-stated findings were reported, an embryo which had shown this hereditary fluid imbalance survived and reached maturity. This animal, a female, spawned a number of times following matings with males heterozygous for the fluid imbalance as well as after matings with males not carrying its gene. The unusual character of her offspring, all of which showed a precocious fluid imbalance whatever their genotype, is being described elsewhere; this constitutes an example of so-called "maternal inheritance," a phenomenon of fairly rare occurrence in animals, resulting from the conditioning of the egg cytoplasm during oogenesis by the mutant genes. The finding of particular interest at this time, however, is that the heterozygous (*Ff*) daughters† of this homozygous (*ff*) female were of two distinctly different types with respect to the nature of their *ff* offspring. Females of the first type, mated with the usual heterozygous (*Ff*) male, produced *ff* offspring having the typical lethal syndrome; females of the second type, similarly mated, produced *ff*, a high percentage of which developed normal gills and proved viable. The viable *ff* females from such spawnings produced offspring with a precocious imbalance such as appeared in the progeny of the first surviving *ff* female (see above), with all embryos of *ff* genotype non-viable because of the extreme distention. The viable *ff* males, mated with the usual heterozygous females (first type men-

*Department of Zoology, Indiana University, Bloomington. This study was begun in the Anatomy Department of the University of Buffalo and continued at Indiana University; the writer wishes to express his gratitude to both institutions for the space and facilities afforded him. The study has also been aided by a grant from the National Science Foundation, whose assistance is gratefully acknowledged. Thanks are due also to Doctors Robert Briggs, G. Fankhauser, and I. Oster for their helpful comments or suggestions.

†This female, being *WW* with respect to sex chromosomes, produced only female offspring.

tioned above), produced offspring of which about half showed the expected fluid imbalance; of these about half proved capable of recovery and survival.

The first interpretation made of these unexpected findings was that one of the *f* genes in the first surviving homozygous (*ff*) female had been altered by mutation in such fashion (say to f^1) that it produced a less serious effect, and that this gene in ff^1 embryos was dominant over the unaltered *f* inducing the usual lethal syndrome. Soon, however, two additional findings compelled the abandonment of this interpretation. It was found that in spawnings from a mating of two of the usual heterozygotes (i.e., those *Ff* not descended from the *ff* female) among the embryos never showing a fluid imbalance, there were usually to be found a small number which exhibited the later features of the imbalance syndrome —peculiarly abnormal gills, failure to feed, and early death. The second finding was that a certain female, which had not shown a fluid imbalance in embryonic life, when mated with a male known to carry the usual or lethal type of gene *f*, produced offspring all of which at first appeared normal (no fluid imbalance) but about one-fourth of which at hatching showed the later features of the lethal syndrome. These findings required the assumption that gene *F* or *f* had undergone still another mutation, or that the lethal imbalance syndrome was actually determined by two linked recessive genes which might occasionally become separated by crossing-over during meiosis. Evidence which shows quite convincingly the correctness of the second interpretation will now be presented.

Separation of the Linked Genes During Meiosis

The lethal imbalance syndrome in the axolotl was originally regarded as determined by a single recessive gene which was designated by *f*. This designation has been retained for the fluid imbalance alone, for which it seems appropriate. The later and actually lethal features of the syndrome (abnormal gills of a specific type, failure to eat, etc.) are the result of a second gene, which, because of the distinctive structure of the gills induced by it, is now being designated by *g*. On this basis, embryos showing the originally discovered lethal syndrome and first designated as *ff* in genotype should be designated as *fg/fg* and their heterozygous parents as *FG/fg* (linked gene pairs in coupling).

In the offspring from a mating of two of the usual heterozygotes (*FG/fg*), 10 genotypes are possible, assuming the loci of *f* and *g* to be far enough apart to permit an occasional crossing-over in both parents. Those genotypes produced without crossing-over (*FG/FG*, *FG/fg*, and *fg/fg*) will constitute the great majority, with the one last named making up most of the 25 percent exhibiting the imbalance followed by abnormal gill development. Among the genotypes resulting from crossing-over, *fG/fg* and *fG/fG* are also homozygous for *f* and hence develop an imbalance, but not being homozygous for *g*, have gills of normal type and are largely viable. The original surviving *ff* female described on page 279 was proved to be of genotype *fG/fg*. Genotypes *Fg/fg* and *Fg/Fg*, on the other hand, are homozygous for *g* but not for *f*; these appear normal in phenotype when the embryos are sorted for the fluid imbalance, but subsequently develop abnormal gills of specific type and never begin feeding. This abnormal phenotype was not detected by the writer in his earlier study because of its small numbers and the fact that *all* embryos first classed as normal were not reexamined at a suitable later stage. Genotypes *FG/Fg*, *FG/fG*, and *Fg/fG* are homozygous for neither *f* nor *g* and hence are phenotypically normal. Their genotypes may be determined, however, by mating them with animals of the parent type, *FG/fg*. The female mentioned previously, none of whose offspring from such a mating showed a fluid imbalance although later one-fourth were found to have the lethal features of the syndrome, was of genotype *FG/Fg*. Genotype *FG/fG*, in contrast, when mated to the parent type, produces offspring about one-fourth of which show

the fluid imbalance without the abnormal gill type and other features determined by homozygosity for gene *g*.

Of the 10 genotypes theoretically possible through the various combinations of genes *F*, *G*, *f*, and *g* (see above), eight have actually been identified either by their distinctive phenotype (the non-viable *fg/fg*) or through test matings with known *FG/fg*. Such test matings for the seven viable genotypes, as shown in Table I, result in progeny showing distinctive percentages of the four easily recognizable phenotypes. The occasional embryos in any test spawning showing other phenotypes due to crossing-over may be disregarded. Embryos of the two remaining genotypes, *Fg/fg* and *Fg/Fg*, are identical in phenotype (lethal type of gills not preceded by a fluid imbalance) and being non-viable, cannot be distinguished by breeding tests.

To identify in a spawning from a mating of the type *FG/fg* × *FG/fg* among the phenotypically normal offspring all those with rare genotypes resulting from crossing-over would necessitate a prohibitive number of test matings. Two genotypes resulting from crossing-over are, however, readily identified by their characteristic phenotypes. Embryos of genotype *fG/fg** develop a fluid imbalance, but since they carry *G*, have normal gills, while embryos of genotype *Fg/fg*, at first apparently normal, eventually show the lethal type of gills resulting from their homozygosity for *g*. Identification of these two relatively rare phenotypes in a sufficient number of spawnings from *FG/fg* parents therefore permits computation of the rate of crossing-over.

Crossing-Over In Matings of Various Types

In Table II are shown the numbers of the two fairly rare phenotypes resulting from crossing-over (see above) as they were identified in spawnings over the years 1956 to 1959 from matings of the type *FG/fg* × *FG/fg*. Of a total of 7,538 in the table not showing a fluid imbalance, 91 had gills of the peculiar type characteristic of *gg* homozygotes. Since without crossing-over, two-thirds of this total or 5,025 should be *FG/fg* and be normal phenotypically, crossing-over of *g* must have occurred 91 times in 5,025, or at a rate of 1.81 percent. Among the 2,655 embryos of this table which did show an imbalance, 120 subsequently developed normal gills. Since gene *g* would have been present 5,310 times in this group of 2,655 embryos if no crossing-over had occurred, the identification of 120 with normal gills (indicating the

TABLE I. Percentages of offspring of the four different phenotypes produced by animals of the seven viable genotypes in test matings with the usual heterozygote, *FG/fg*. Crossing-over disregarded.

Genotype tested	Types of offspring			
	Normal phenotype	Imbalance, gills lethal type	Imbalance, gills normal type	No imbalance, gills lethal type
FG/FG	100%	—	—	—
FG/fg	75	25	—	—
FG/fG	75	—	25	—
FG/Fg	75	—	—	25
*fG/fg**	50	25	25	—
*fG/fG**	50	—	50	—
Fg/fG	50	—	25	25

*Only the *males* of these two genotypes may be identified and distinguished as indicated. The precocious and severe imbalance in the *ff* offspring of females of both genotypes alike precludes all possibility of classification of their gill types. Females of the two genotypes may be distinguished by ascertaining the genotypes of a number of their viable offspring heterozygous for *f*.

*Genotype *fG/fG*, which would have this same phenotype, results from the union of two gametes produced by crossing-over, and with this at the low rate here found (about two percent), would be so extremely rare (about one in 10,000) as to be negligible. Genotype *Fg/Fg* would produce the same phenotype as does *Fg/fg*, but may likewise be disregarded because of its extreme rarity.

presence of *G*) gives a rate of crossing-over for this group of 2.26 percent. The difference between this and the lower rate of 1.81 percent based on the group not showing an imbalance is probably in large part accounted for by the fact that embryos with an imbalance but developing normal gills may be identified with more certainty than those with gills of the lethal type among embryos previously normal. Embryos cannot be classed as having gills of the lethal type unless they are free of defects (such as microcephaly, lack of a circulation, etc.) which might have produced gills somewhat similar to the lethal type. Merely abnormal gills must be disregarded; the abnormality in the genetically determined lethal type consists (a) in the fewer and more variable filaments, producing a somewhat antler-like appearance as contrasted with the pinnate form of the normal gills, (b) the tendency of the gill tips to bend forward, especially those of gill 3, (c) the subnormal size of the gills, and (d) their tendency to show distention in their proximal portions. Criteria (a) and (b) are most important in recognition of this gill type. The normal type of gill in embryos showing the imbalance, on the other hand, is more often recognizable in embryos with microcephaly, absence of circulation, or other abnormalities.

The viability of males of genotype *fG/fg* makes possible another type of mating from which the rate of crossing-over may be determined. This mating (*fG/fg* male × *FG/fg* female) produces offspring about one-half of which show a fluid imbalance (genotypes *fg/fg*, *fg/fG*, and *fG/fG*, the last plus a few of the *fG/fg* being the result of crossing-over in the female), and about one-half of which lack the imbalance (genotypes *FG/fg*, *FG/fG*, *fG/Fg*, and *Fg/fg*, the last two also the result of crossing-over during oogenesis). Genotype *Fg/fg*, being homozygous for *g*, may be distinguished from the others of the non-imbalance group when it develops gills of the lethal type.

In Table III are included 2,578 embryos showing no imbalance obtained from matings of the type just described; those embryos showing an imbalance have been omitted. Without crossing-over, half of the 2,578 or 1,289 should have been *FG/fg*, but since 27 showed the lethal type of gills and must be *Fg/fg*, crossing-over of *G* and *g* in the ova at a rate of 2.09 percent must have occurred.

The identification of a female of genotype *FG/Fg* (see page 280 and Table I) permitted a mating one-fourth of the

TABLE III. Offspring of *FG/fg* female mated with *fg/fG* male

Year	Gill types in embryos not showing fluid imbalance		
	Normal	Lethal type	Unclassed*
1956	755	7	62
1957	1,445	18	65
1958	208	2	16
Totals	2,408	27	143

*Gills of abnormal type but not the characteristic lethal type due to *gg*, or lacking sufficient development to permit classification.

TABLE II. Offspring from matings of *FG/fg* × *FG/fg*

Year	Gill types in embryos not showing fluid imbalance			Gill types in embryos with fluid imbalance		
	Normal	Lethal type	Unclassed*	Normal	Lethal type	Unclassed*
1956	1,967	23	164	35	716	**
1957	2,685	41	105	44	819	68
1958	1,197	10	75	19	450	42
1959	1,179	17	75	22	350	90
Totals	7,028	91	419	120	2,335	200

*Gills of abnormal type but not the characteristic lethal type due to *gg*, or lacking sufficient development to permit classification.

**Included in lethal type.

progeny from which carried genes *f* and *g* in different chromosomes (linked genes in repulsion). This mating (*FG/Fg* female × *fg/fG* male) produces progeny of four genotypes, *FG/fg*, *FG/fG*, *Fg/fg*, and *Fg/fG*, none homozygous for *f* and having a fluid imbalance, but one (*Fg/fg*) which is non-viable because of its homozygosity for *g*. The one having the linked genes in repulsion (*Fg/fG*) thus constitutes one-third of the viable progeny. It may be readily distinguished from animals of the other two viable types by test matings with animals of genotype *FG/fg* (see Table I). While this new genotype may be encountered in the progeny of parents both *FG/fg* in genotype, its occurrence in such spawnings requires the union of two gametes (*Fg* and *fG*) both resulting from crossing-over. With this at a rate of two percent, only about two in 10,000 progeny may be expected to be of genotype *Fg/fG*, as contrasted with the 25 percent in spawnings of *FG/Fg* females mated with *fG/fg* males.

Since the various findings thus far cited led to the conclusion that the imbalance with lethality as originally discovered is really a syndrome determined by two linked genes, it became of interest to ascertain whether the genotype essential for this complete syndrome could be reconstituted in the progeny of animals of genotype *Fg/fG* in which such linkage has been broken by crossing-over. In other words, will crossing-over occur to restore the original *f-g* linkage?

From a mating of an *Fg/fG* female with an *fG/fg* male, the genotypes produced without crossing-over in the female are *Fg/fG*, *Fg/fg*, *fG/fG* and *fG/fg*, the last two, homozygous for *f*, constituting almost half of the spawning. Not being homozygous for *g*, however, their gills are of normal type. If crossing-over occurs in the *Fg/fG* parent, a few embryos of each of four genotypes result: *FG/fG*, *FG/fg*, *fg/fG*, and *fg/fg*. The last, being homozygous for *g* as well as *f*, will differ from the other embryos showing the imbalance in that it develops the lethal type of gills as in the original syndrome.

Thus far 235 embryos with fluid imbalance have been obtained from matings of the type just described. Of these, six had gills definitely of the lethal type expected in *gg* embryos; this would give a recombination rate of 2.55 percent.

In spawnings of *Fg/fG* females mated with *FG/fg* males, one-fourth of the progeny show a fluid imbalance. These are usually of the genotype *fG/fg* or *fG/fG* and develop normal gills. By crossing-over in the female parent, however, a few gametes of *fg* type are produced, resulting in progeny of genotype *fg/fg* which exhibit an imbalance but develop the lethal type of gills. From 14 matings of this type, 674 embryos with a fluid imbalance were obtained of which 20 developed the lethal type of gills determined by homozygosity for *g*; this would indicate crossing-over at a rate of 2.97 percent. From matings of the reciprocal type (*Fg/fG* male × *FG/fg* female) 860 embryos with imbalance were obtained, of which 14 were cross-overs (gills of lethal type). The rate in this group is only 1.63 percent, considerably lower than that in the two groups in which crossing-over had occurred in the female (2.55 and 2.97 percent). For the three groups combined there are 35 recombinations in 1,477 embryos, or a rate of 2.37 percent. This is fairly close to the rates of crossing-over by which separation of *f* and *g* occurred in the animals of *FG/fg* genotype in which these linked genes were first discovered (rates of 1.81, 2.26, and 2.09 percent; see Tables I and II). Combining the data on which these three rates are based, the total number of cross-overs would be 238 in 11,624 or 2.05 percent. Taking into account all the data, one may conclude that crossing-over to restore the *f-g* linkage differs little in rate from that by which *f* and *g* undergo separation.

The matings of *Fg/fG* animals demonstrate that crossing-over occurs in gametogenesis in the male as well as in the female. Although the data suggest a somewhat higher rate in the latter sex, this difference may be shown to be apparent rather than actual as additional data are accumulated.

Absence of Gills of Lethal Type In Spawnings of *FG/FG* × *FG/fg*, *FG/FG* × *FG/FG*, etc.

To ascertain whether the complex supposedly determined by gene *g* (lethal type of gills, failure to eat, etc.) occurs in spawnings in which, from the known genotypes of the parents, it should, theoretically, not appear, careful examination was made at the proper time of all embryos from numerous matings of many types.

In Table IV, of a total of 4,892 progeny from matings of *FG/FG* with *FG/fg* none was found to have gills of the lethal type. Nor could gills of this type be positively identified in any of over 9,600 offspring from other matings of parents one or both of which were known to be of genotype *FG/FG*. In short, this characteristic gill type has thus far been identified with certainty only in spawnings for which *both* parents appear to carry the gene for it (*g*).

One other type of mating of some interest may be noted: that of animals proven by test matings to be of genotype *FG/Fg* with animals similarly determined to be of genotype *FG/fG*. As expected, the embryos in the resulting spawnings (163 in all) included none showing either a fluid imbalance or gills of the lethal type.

Discussion

The findings here reported all appear in accord with the conclusion that the lethal imbalance syndrome of the axolotl embryo as originally reported[1] is not determined by a single gene but by two recessive genes occupying loci fairly close together in the same chromosome. One of these, *f*, in embryos homozygous for it, results in the fluid imbalance of earlier embryonic life, a condition not necessarily lethal although often proving so because of the damage from persistent severe distention. The second gene, *g*, is invariably lethal in the homozygous condition; while its exact mode of action has not as yet been determined, its outstanding structural manifestation is the development of abnormal gills of a specific type.

Since crossing-over occasionally separates these two genes, resulting in phenotypes in which the traits they determine appear separately, the failure to discover these during the earlier study requires a word of comment. (1) In that study, after the embryos were sorted into two groups, one "normal," the other showing the imbalance, not *all* embryos of the first group were subsequently re-examined under the microscope; the inspection of limited samples from this "normal" group permitted the small numbers which later developed abnormal gills to escape notice. (2) Since food was offered the "imbalance" group at the proper time, it may be asked why those of this group developing normal gills and becoming capable of feeding and survival did not attract more attention. Here two or three factors are involved: (a) many *ff* larvae not homozygous for *g* and therefore developing more or less normal gills may be otherwise damaged through the distention they suffer and so fail to eat and survive; (b) at the time of the previous study, larvae were being given Enchytraeus (white worms) as their first food; with these, more larvae of entirely normal type fail to begin eating than when they are started on smaller organisms such as newly hatched Artemia; and (c) those few affected larvae which did eat and survive for a time could be logically assumed to have done so because of having suffered less damage to the gills from the early distention involving the branchial region.

It is, of course, of great theoretical interest that two separately determined manifestations, fluid imbalance and lethality with abnormal gills, should have been found comprising a syndrome readily interpretable as due to a single recessive gene. Whether the two mutations involved occurred simultaneously or at different times is a pertinent question still unanswered. The writer knows of no published description of either mutation alone or of the two in combination prior to his original publication in 1948. The one certainty is that among the white axolotls of the Wistar Institute colony in 1935 there was one, a female given to the writer, which carried the two mutant genes in the linked condition (genotype *FG/fg*). We may only speculate as to how they attained this relationship.

TABLE IV. Offspring from matings of *FG/FG* × *FG/fg*

Year	Gill types in embryos (none showing fluid imbalance)		
	Normal	Lethal type	Unclassed*
1956	1,158	0	71
1957	2,449	0	70
1958	249	0	6
1959	824	0	65
Totals	4,680	0	212

*Includes those with gills of abnormal type though not the characteristic lethal type due to *gg*, and those lacking sufficient gill development to permit classification.

Assuming that in the white strain of the Wistar Institute, or the European stock from which it was derived, mutations to *f* and *g* had occurred independently, producing animals of genotypes *FG/fG* and *FG/Fg*, it would be theoretically possible, through two successive matings of the proper type, to produce an animal of genotype *FG/fg*. From a mating of two heterozygotes, one of which carried gene *f*, the other *g*, one-fourth of the offspring would be of genotype *fG/Fg*. In random matings of these, the occasional gametes carrying *f* and *g* in the same chromosome as a result of crossing-over would produce limited numbers of individuals with the genotype *FG/fg*. If genes *f* and *g* had attained a fairly high frequency in the population involved, permitting numerous matings of the first type postulated (*FG/Fg* × *FG/fG*), animals of genotype *FG/fg* would eventually appear in considerable number, although still constituting but a small fraction of the total population.

It is possible, of course, that the two mutations might have occurred in succession, in such fashion that the linkage discovered was established at the occurrence of the second mutation. Had gene *f* arisen first, however, and become fairly widely distributed before a mutation to produce *g* occurred in a chromosome already carrying *f*, the chances would seem to favor the perpetuation of *f* in the separate rather than the linked condition, since the *fg/fg* homozygotes are always non-viable while many of the *fG/fG* could survive. Again the chances would seem to be against an animal selected at random proving to be *FG/fg* rather than a genotype having *f* alone. Had gene *g* been the first of the two mutations to occur, a subsequent mutation of *F* to *f* in a chromosome already carrying *g* would have given the linkage as discovered; the linked genes, however, should have no greater survival value than *g* alone, since the *fg/fg* genotype, like the *Fg/Fg*, is always non-viable. Through crossing-over, gene *f* would shortly be present (though in small numbers) not linked with *g*, and would have a somewhat better chance to increase under that condition. Thus again there would appear to be little reason to anticipate animals with the two mutants linked becoming more abundant than those having one of these genes alone.

While it would have been possible for the *f-g* linkage to have come into existence in one of the fashions postulated, it seems more probable that it may have arisen through the more or less simultaneous occurrence of the two mutations in one chromosome. There is no reason to assume that a mutagenic agent capable of inducing mutation at one locus could not have affected a neighboring locus at approximately the same time. Following such an origin, the linked genes could have become separated through crossing-over, but the low rate at which this occurs, due to their proximity in the chromosome, would tend to insure their preponderance in the linked condition for many generations.

Unfortunately we have no adequate information available concerning the occurrence of genes *f* and *g* in colonies of the white axolotl now maintained in Europe. Brunst* states that he recalls encountering white axolotl embryos with a fluid imbalance in a laboratory in the Ukraine many years ago; this white stock may have been closely related to that in Cracow, Poland, from which the first white animals of the Wistar Institute colony were obtained. The writer has had no opportunity to test animals from European laboratories with the exception of a single male† from the colony of Dr. Hans Gloor in Zurich; this animal carried neither *f* nor *g*.

Whether or not the two mutations may have arisen simultaneously at some time in the past, we have no convincing evidence that they have done so in the more recent period in which they have been under study. Nor do we have evidence that either mutation has occurred separately during this period. In a few instances embryos having the typical fluid imbalance have been encountered in spawnings from matings of the type *FG/FG* × *FG/fg*, but these have usually been explainable on a basis other than mutation of *F* to *f* in a germ cell of the homozygous parent. Some such embryos have been proven to be haploids carrying a chromosome set from the heterozygous parent; some of the others, usually from eggs subjected to refrigeration, appeared to be hypo-diploids in which loss of the chromosome carrying the dominant gene *F* had permitted the expression of its recessive mutant. All such embryos have been non-viable, making impossible a positive identification of their genotypes through breeding tests. With animals now available carrying gene *f* without a linked *g* (genotypes *FG/fG* and *fG/fG*), a mutation of *F* to *f* might be more readily detected. A mutation of *G* to *g* will remain difficult or impossible to demonstrate satisfactorily since the embryo homozygous for *g* never survives.

The separation of genes *f* and *g* and the consequent availability of embryos homozygous for one or the other alone, make possible experimental studies designed to test the modes of action of these two mutants. Thus it has been found that the fluid imbalance in embryos homozygous for *f* may be alleviated or corrected through parabiosis with a normal embryo. Appropriate experiments with embryos homozygous for *g* may eventually serve to re-

*Personal communication.

†Obtained through the kindness of Dr. H. C. Dalton of New York University.

veal more completely the nature of its lethal effects.

Summary and Conclusions

The fluid-imbalance syndrome in the Mexican axolotl, first interpreted as a simple Mendelian recessive, has been demonstrated to be determined by two mutant genes occupying loci close together in the same chromosome. One gene, designated by *f*, determines the fluid imbalance, which is not necessarily lethal, though often proving so because of the distention resulting from the excess fluid. The linked gene, designated by *g* because of the distinctive abnormality of the gills in embryos homozygous for it, is invariably lethal.

Matings of animals heterozygous for these linked genes (genotype *FG/fg*) have demonstrated that they may become separated by crossing-over during meiosis, and that this occurs at a rate of approximately two percent.

Through appropriate matings several animals have been obtained in which genes *f* and *g* are both present but in the allelic arrangement of "repulsion" (genotype *Fg/fG*). Matings of these with animals of genotype *FG/fg* or *fG/fg* demonstrate that crossing-over during meiosis occurs in both sexes alike to restore the original *f-g* linkage; the frequency of recombination is again about two percent.

Of the 10 genotypes theoretically possible through various combinations of *F*, *G*, *f*, and *g*, the seven of viable phenotype have all been identified through the character of their progeny in test matings. One genotype, *fg/fg*, always lethal, is readily identified by its distinctive phenotype. The remaining two genotypes, *Fg/Fg* and *fg/Fg*, are alike in phenotype, and since both are invariably non-viable, cannot be distinguished by breeding tests.

Since separation of mutants *f* and *g* through crossing-over has provided breeding stock of the necessary genotypes, it is now possible to obtain embryos homozygous for either gene alone. With these it may be possible through experimental procedures to determine more accurately the mode of action of each of these mutants.

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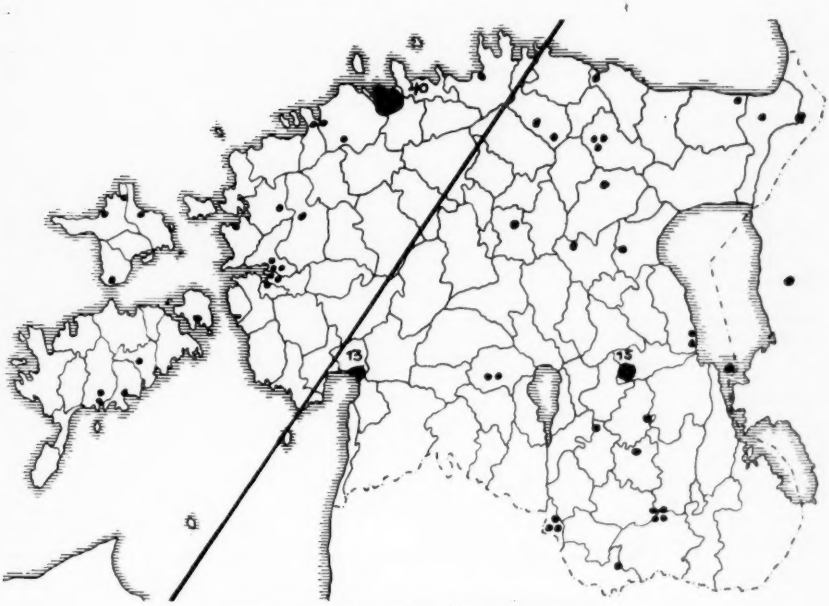
BLOOD GROUPS OF ESTONIANS

A_1 A_2 B O and Rh

LARS BECKMAN AND PER KALLSTENIUS*†



DISTRIBUTION OF TESTED INDIVIDUALS

Figure 13

Distribution in Estonia of the birth-places of the tested individuals. Bigger dots with figures indicate many individuals. The line divides the sample into two subsamples.

THE East Baltic peoples are known to be serologically different from other European populations, the *B*- and *M* frequencies being relatively high as compared with those of western Europe. Thus the Gulf of Bothnia and the Baltic are looked upon as a serological border (Boyd[2] and Mourant[5]).

Concerning the frequencies of the A_1-, A_2-, and *Rh*-subgroups we have information for the Finns (Mustakallio[6] and Nevanlinna[7]) and the Latvians (Race *et al*[8]), but information from Estonia has not been obtained before as far as we know. The A_2-frequency in Latvians was found to be only 2.5 percent, which is significantly lower than in Finland (Table I). The southwestern part of Finland shows a somewhat higher A_2-frequency than the southeastern part of the country, which, according to Mourant, is possibly due to a more pronounced Lappish influence in southwestern Finland.

Materials and Methods

Finger-prick specimens of blood from 151 Estonian immigrants were taken into tubes as heparinized blood and typed within 18 hours.

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The anti-A_1- and -H- sera were kindly supplied by Professor B. Broman of the Blood Group Serological Department of the State Laboratory for Forensic Chemistry in Stockholm.

The birth places of the blood donors are shown on Figure 13. The material has been divided into two samples according to the dividing line seen on the map.

Gene frequencies have been calculated by the methods recommended by Mourant[5].

Results and Discussion

a) A_1 A_2 B O

The results are shown in Tables II and III. The consistency of the material is satisfactory. The frequencies show a fairly good agreement with earlier A-B-O-data from Estonia (cf. Boyd[2]) and A_1-A_2-B-O-data from Latvia (Table I). Earlier investigations have shown the B-frequency to be lower in the western part of Estonia. In spite of the small material, the same condition is demonstrated by the present data.

The heterogeneity for $(B+AB)$ between the eastern and western samples gave $\chi^2 = 4.81$, 1 d.f., $0.05 > P > 0.02$. The frequency of $(A+AB)$ was shown to be significantly higher in the western sample ($\chi^2 = 4.09$, 1 d.f. $0.05 > P > 0.02$). All A_2-individuals belonged to the western sample.

The A_2-frequency is significantly lower than that of Finland ($P < 0.001$); hence, it seems that the Gulf of Finland represents a serological border as to the A_2-frequency. The Estonian population has the same origin as the Finnish one to a large extent. The Lapps are known to have spread over all of Finland in earlier times, but not as far as Estonia (Collinder[4]). Thus the rise of A_2 frequencies coincides with the archaeologically known dispersion area of the Lapps. It seems therefore probable that the non-Lappish part of the Finish population did not originally possess its present relatively high A_2 frequency, but, as Mourant[5] already has proposed, has attained it through a later intermixing with the Lapps, above all in the southwestern part of the country.

b) *Rh*

The frequencies of the *Rh*-subgroups are shown in Table IV. The frequencies of *Rh*- and *D*-negative individuals are comparatively high. No significant difference was found between the two samples.

The frequencies of the *C*- *D*- and *E*-genes (Table V) did not differ to any remarkable extent from those of the adjacent populations.

The frequencies of the *CDe*- and *cDE*-chromosomes seem somewhat lower as compared with those of Sweden and Latvia, while the *Cde*-, *cDe*- and *cde*-chromosomes are relatively high (Table VI).

TABLE III. A_1A_2BO gene frequencies of Estonians

	p_1	p_2	q	r	p_1+q_1+r
Western part	22.8	5.5	11.9	59.8	99.31
Eastern part	17.2	—	20.8	62.0	98.63
Total	20.9	3.4	14.4	61.3	98.16

TABLE I. A_1A_2BO gene frequencies and frequency of *Rh*-negatives in Lapps, Finns and Latvians

Population	Investigator	A_1	A_2	B	O	% *Rh*—
Swedish Lapps	Allison *et al.*[1]	4.51	32.33	2.09	61.07	3.63
Finns	Mustakallio[6] Nevanlinna[7]	20.9	8.6	12.3	58.2	12.8
Latvians	Race *et al.*[8]	25.96	2.51	14.98	56.55	13.37

TABLE II. A_1A_2BO groups of Estonians

		A_1	A_2	B	A_1B	A_2B	O	n
Western part	n	36	7	16	3	1	34	97
	%	37.11	7.22	16.50	3.09	1.03	35.05	
Eastern part	n	14	—	17	3	—	20	54
	%	25.93	—	31.48	5.56	—	27.04	
Total	n	50	7	33	6	1	54	151
	%	31.11	4.64	21.85	3.97	0.66	35.76	

Summary

A_1A_2BO- and *Rh(CDE)*-blood groups of 151 Estonian immigrants have been determined.

A comparatively low *A_2*-frequency was found in the Estonians in accordance with earlier results concerning Latvians. The frequency of *Rh*-negative individuals was relatively high.

In spite of the small material, significant differences concerning the *A*- and *B*-frequencies could be demonstrated between eastern and western Estonia.

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TABLE IV. *Rh* groups of Estonians

		CDE	*CD*	*DE*	*CE*	*C*	*D*	*E*	*cde*	n
Western part	n	9	46	15	—	6	4	—	17	97
	%	9.28	47.42	15.46	—	6.19	4.13	—	17.53	
Eastern part	n	9	27	5	—	2	—	2	9	54
	%	16.67	50.00	9.26	—	3.70	—	3.70	16.67	
Total	n	18	73	20	—	8	4	2	26	151
	%	11.92	48.34	13.24	—	5.30	2.65	1.33	17.22	

TABLE V. Frequencies of the *Rh* gene

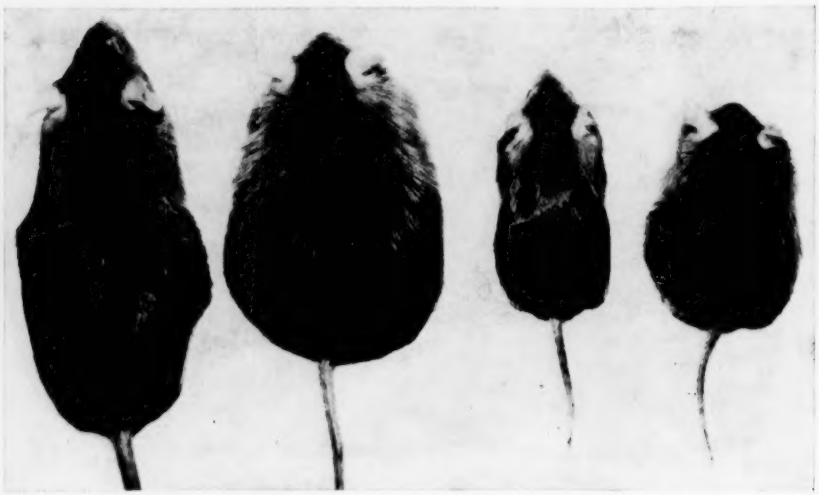
D	*d*	*C*	*c*	*E*	*e*
51.2	48.8	41.3	58.7	14.3	85.7

TABLE VI. Chromosome frequencies

Sample	*CDe*	*cDE*	*cDe*	*Cde*	*cdE*	*cde*	n
This investigation	0.353	0.127	0.031	0.059	0.016	0.414	151
Latvians, Race *et al.*[8]	0.4295	0.1662	0.0078	0.0230	0.0078	0.3657	172
Swedes, Broman[3]	0.4155	0.1590	0.0178	0.0144	0.0080	0.3852	2768

ADIPOSE, A NEW INHERITED OBESITY OF THE MOUSE

D. S. Falconer and J. H. Isaacson*



COMBINATION OF ADIPOSE AND PITUITARY DWARF

Figure 14

Left to right are shown normal, adipose, dwarf and adipose dwarf mice.

THIS gene arose spontaneously in a strain selected for large size. The strain originated from a cross between Goodale's and MacArthur's Large Lines, and was subsequently selected for increased growth from three to six weeks. In the ninth selected generation, two litter-mate females grew to be excessively fat, one reaching 100 gm. in weight at four months, when the weight of normal females of the strain was about 50 gm. The abnormal fatness reappeared sporadically in subsequent generations. Since it appeared always in about one-quarter of the offspring of certain matings, it had the appearance of being due to a single recessive gene, and subsequent tests proved this to be so, and proved further that the gene responsible is not allelomorphic with the already known gene obese (*ob*)[1]. The new gene will be called *adipose*, with the symbol *ad*. The gene was extracted from the strain of origin by means of crosses to unrelated strains and was subsequently studied in a background of normal size.

Description

Adipose homozygotes are indistinguishable from normals up to the age of three weeks. At about four weeks they begin to appear fatter, but they are not classifiable with certainty until five or six weeks of age, when they are clearly

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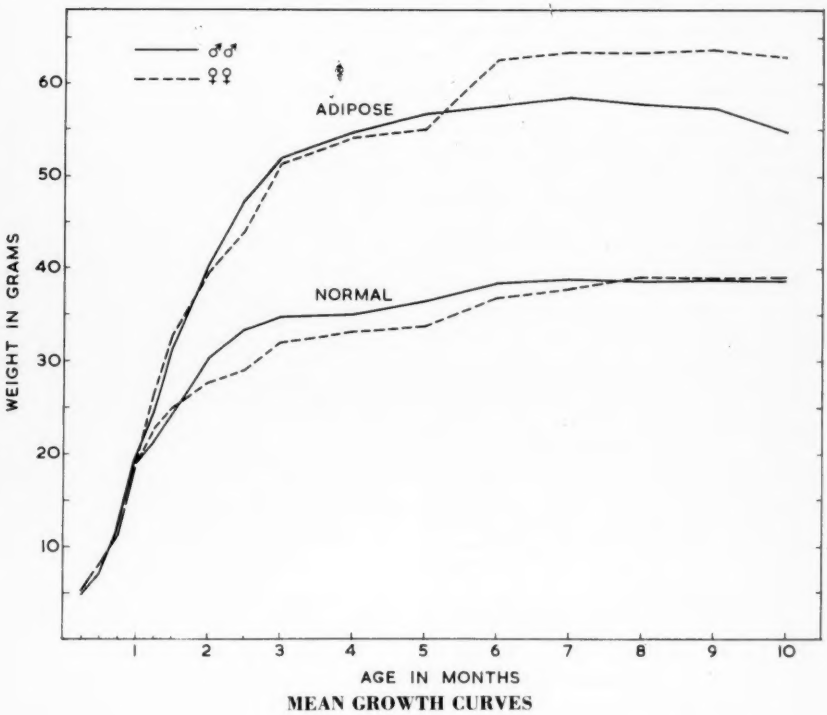

MEAN GROWTH CURVES

Figure 15

Mean growth curves of adipose homozygotes and normal litter-mates. The graphs of females are each based on five animals drawn from three litters; those of males on three animals from two litters. The mice were weighed weekly up to six weeks, then at 8, 10 and 12 weeks and thereafter at monthly intervals. The weights of the four groups up to four weeks could not be distinguished in the graphs. Three adipose females and two normal females died between five and six months. The survivors were heavier than those that died, and this accounts for the apparent increase of weight, seen particularly in the adipose females.

differentiated in weight as well as in appearance. Growth curves of adipose homozygotes and normal litter-mates are shown in Figure 15, and an adult adipose is pictured in Figure 14. All attempts to breed from homozygotes of either sex have failed. Tests of fertility, however, were not made systematically—probably not more than 10 of each sex were tested—and so complete sterility was not proved. Adipose homozygotes are indistinguishable from obese homozygotes by superficial examination, but no physiological studies have been made. Heterozygotes were not specifically studied, but it is improbable that they differ in weight from normal homozygotes because the gene decreased in frequency in the strain of origin despite continued selection for large size.

Genetics

Individuals proved to transmit adipose produced no adipose progeny when outcrossed to unrelated stocks. Altogether 109 outcross progeny were examined (including the tests with obese described below). This proves the gene to be recessive. Proof that adipose is a single gene was obtained from the following segregation among the offspring of matings between heterozygotes (+/*ad* ♀ × +/*ad* ♂):

No. of matings	*Normal*	*Adipose*	*Total*	*% adipose*
19	292	116	408	28.4±2.2

The observed frequency of adipose was 28.4±2.2 percent, and does not differ significantly from the expected 25 percent. ($\chi^2_{(1)} = 2.56$; P ~ 0.1). There was some bias in the data, introduced by the exclusion of matings which may have been between heterozygotes but which produced no adipose offspring, and so the true expectation was somewhat greater than 25 percent. These data came from 19 matings, which were substantially homogeneous in their ratios. When four matings with fewer than 10 classified progeny were excluded, the test of heterogeneity gave $\chi^2_{(14)} = 20.3$; P ~ 0.1.

Thus the observed segregation of adipose points to a single recessive gene with complete penetrance and full viability up to the time of classification.

Tests of identity with obese (*ob*) were made by mating proved adipose heterozygotes with proved obese heterozygotes, obtained from a stock kindly supplied by Dr. G. A. Harrison. Crosses were made between several different individuals in order to remove any possibility of error from mistaken identities. Altogether 72 testcross progeny were reared, and all were classified as normal. The progeny were distributed as follows: 12, 26, and 14 respectively from one +/*ob* ♂ mated to three different +/*ad* ♀♀; 9 from another +/*ob* ♂ by another +/*ad* ♀; and 11 from a +/*ob* ♀ by a +/*ad* ♂. If adipose were identical with, or an allele of, obese, one quarter of the progeny would have been abnormal. The absence of any abnormal progeny among this large number conclusively disproves the identity of the two genes.

Very little information about the linkage of adipose was obtained, but close linkage with sex and with leaden (*ln*) was excluded. The observed recombination between adipose and sex was 37 percent ± 10 percent, and between adipose and leaden was 41 percent ± 10 percent.

Interaction with Pituitary Dwarf

A double homozygote for adipose and pituitary dwarf (*dw*) appeared (more by accident than by design) in the progeny of a stock mating. This animal is shown in Figure 14, along with the two single homozygotes and a normal mouse. The dwarfism does not interfere with the adiposity, nor does the adiposity affect the skeletal growth of the dwarf.

Summary

Adipose (*ad*) is a new gene causing obesity. It is recessive and fully penetrant. Homozygotes are indistinguishable in external appearance from obese (*ob*) homozygotes, but the two genes are not allelomorphic.

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MULTIPLE SIRE MATING IN SWINE

I. General Considerations

LAVON J. SUMPTION, WILLIAM E. REMPEL, AND LAURENCE M. WINTERS*

SYSTEMS of mating have received major attention in swine breeding research in the attempt to discover effective methods for the genetic improvement of economic traits. Early investigations involved various regular systems of mating (e.g., brother-sister, half brother-sister, double first cousin, etc.). More recently several breeds have been developed by conventional pedigree mating of each dam with a specific sire without special regard to relationship, i.e., "flexible" inbreeding[19] or "irregular" inbreeding[11].

The Minnesota No. 3 line of swine was initiated using multiple sire mating[18]. From each generation, a number of sires were carefully selected on the basis of individual performance and performance of contemporary litter mates. The sires as a group were mated with the selected dams. No attempt was made to control the matings that occurred. Because this is a relatively new approach in the development of improved breeds, it is pertinent to examine some of the possible values of multiple sire mating in swine breeding. Further studies are being conducted at the Nebraska Experiment Station for later publication in this series.

Literature Review

Darwin emphasized the importance of competition between sires to promote natural selection for effective mating[12]. The only previous published reports of the extensive use of multiple sire mating are those dealing with the development of new breeds of cattle. In establishing the Santa Gertrudis, breeding herds have ranged in size from five cows and a single bull to 1700 cows and 70 bulls[13]. Multiple sire mating has been used throughout the period of development of the Beefmaster cattle[9]. The specific contribution of multiple sire mating as a breeding system was not evaluated experimentally, but both projects have led to development of productive cattle adapted to range conditions of southwestern United States. Previous studies show that females of multiparous species can produce progeny by more than one sire in a single litter, as demonstrated in mice by Edwards[3]; furthermore, early crossbreeding studies in swine were conducted by "double mating" sows of breed A with boars of breeds A and B to produce purebred and crossbred pigs in the same litter[14].

Materials and Methods

Observations were made in the early development of the Minnesota No. 3 line of swine, the foundation of which includes some contributions from 12 breeds[18]. The major objective of the project was to study methods of developing breeds with high performance as parent lines and consistently high, uniform performance in crossbred combinations. Multiple sire mating was adopted as a procedure that might be useful in accomplishing the objective.

Controlled matings were made to produce F_1 litters. During five succeeding generations the majority of matings were made by lot breeding nine to 12 boars with 50 to 75 gilts. No attempt was made to regulate order or frequency of mating; boars remained with

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the gilts at all times during the mating period. As a result, it was theoretically possible for each female to mate with all of the sires. All sires were removed after a single estrus cycle and gilts that did not conceive were discarded. From the F_2 through the F_4, gilts were mated to produce progeny at less than 12 months to place selection pressure on early sexual maturity.

The No. 3 line has been pedigreed primarily from the female side. All sires were included as a group on each pedigree. The only accurate identification of progeny groups that was maintained was according to dam "families."

Results

Early attainment of sexual maturity was evident after the F_1. In each succeeding generation litter-mate matings occurred frequently before completion of performance testing at 140 days. Pregnancy seldom ensued. However, the majority of gilts were cycling regularly by 160-180 days of age.

General observations of mating behavior were made each year at breeding time. Sires that exhibited abnormal mating behavior or lack of sex drive were removed from the herd immediately. Limited data were taken during part of the first two days of breeding in 1957. Ten boars were placed in the same lot with 75 gilts. One boar was removed when it became obvious that an adhesion prevented effective copulation. Data on the frequency of mating during the periods of observation are presented in Table I.

It will be noted that in two days gilt A mated with seven of the nine available boars a total of 12 times during the observation periods. Gilt D was mated 10 times by eight sires. The sires as a group were alert, aggressive breeders. Competition among sires was observed and played a role in order of mating, particularly when the males were grouped about one or more females, but was not an obvious factor when the females in estrus were distributed throughout the mating area. Competition did not prevent the more timid sires from mating, although they seldom were first to mate, nor did they mate as frequently.

In a majority of litters produced in this population, the within-litter variation in superficial traits suggested that progeny of more than one sire might be represented. Litters could ordinarily be divided into two or more subgroups on the basis of color and body form. Undoubtedly, some of these differences may have been due to segregation where the litter was sired by only one boar, but they occurred too frequently for this to be the only explanation. However, detailed analysis of a complex of superficial traits, after Anderson[1], or serological tests would be required to provide proof of multiple paternity in the advanced generations.

Discussion

General

Conventional pedigree breeding involves mating each female with a specific male (single sire mating). The population consists of as many combinations of random samples of gametes from the respective parents as there are progeny produced. In the case of each individual the probabilities involved include the chance combination of genes that segregate to the functional gametes in gametogenesis, the likelihood of a particular sperm and egg reaching the site of fertilization, the probability of a sperm of a specific genotype fertilizing an egg of another specific genotype, and finally the survival of the zygote.

Under single sire mating, the breeder has no control over the specific gene combinations generated either at gametogenesis or fertilization. Furthermore, the breeder has no assurance that it will be possible to obtain a similar genotype by repeating the same mating. Therefore, it is proposed that multiple sire mating represents a conservative extension of the probabilities involved in conventional pedigree breeding. A heterogeneous population of gametes from a group of sires are substituted for the heterogeneous population of gametes from a single sire. Under either system of mating, the breeder must accept for study those genetic combinations that occur by chance. It should be clear that with multiple sire mating there is no sacrifice of the opportunity to exercise care in the selection of sires even though specific matings are not made. If performance data are used as the primary criteria for selection of the phenotypically superior parents within a generation, it is questioned whether the breeder can predict the

TABLE I. Observation of multiple sire mating, generation 5, Minnesota no. 3

Gilts	Day I (4 hour period)		Day II (3 hour period)*	
	No. of Boars	No. of Matings	No. of Boars	No. of Matings
A	6	11	1	1
B	5	8	1	1
C	4	8	2	2
D	6	8	2	2

*All different pairings from Day I.

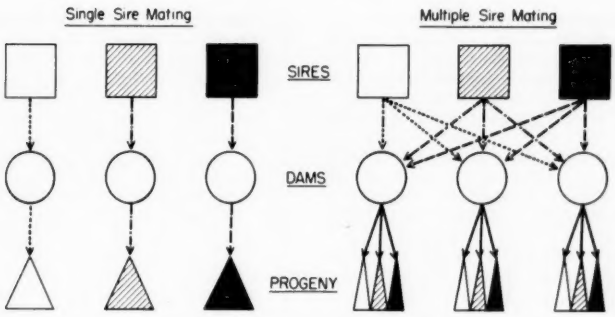

Figure 16
A contrast of potential recombination under single sire and multiple sire mating.

outcome of individual matings well enough at the present time to justify assignment of dams to specific sires. This is especially true for the traits with lower heritability values under mass selection.

One reason for adopting multiple sire mating was to augment simplicity of handling a larger population of females than had been bred in the development of previous lines. It has proved satisfactory in this regard. The limited data also indicate that females in estrus are mated with sufficient frequency to allow a favorable opportunity for the fertilization of all ova that are shed at a physiologically optimum time.

Natural Selection

It is assumed that the more aggressive males should tend to sire more progeny, which would be available for subsequent selection. Because there is evidence suggesting that sex drive has a genetic basis[5, 10], it may be logical to assume that natural selection for aggressive mating behavior in the male may lead to more favorable endocrine relationships that eventually might be beneficial to the improvement of fertility in both sexes. On the other hand, it is also possible that the more aggressive breeders may experience periods of temporary sterility during which less active sires may impregnate more females than might be expected by general observations of mating behavior. Nevertheless, it appears that the possibility exists for natural selection to favor the more active sires which are capable of prolonged production of viable sperm.

Recombination

Successful genetic improvement depends to a considerable extent upon utilizing a system of breeding that will make new gene combinations available for selection in relatively large numbers. Swine breeders are always restricted to the study of relatively small populations considering the theoretically possible chromosome combinations that could be generated (quite aside from considering crossing-over and possible effects of chromosome aberrations). Therefore, it is pertinent to consider a breeding procedure that could make a greater variety of gene combinations available in breeding programs where population size is a major limiting factor. Potential recombination will be contrasted under (1) single sire and (2) multiple sire mating.

Under single-sire pedigree mating, each female is assigned to a specific sire on some arbitrary basis (performance data, pedigree, type, randomization, etc.). Assignment using performance data is somewhat arbitrary, even where "corrective mating" is the object because it is carried out at the phenotypic level with no assurance of genotypic success. If the genotypes of the dam and the sire to which she is assigned fail to combine favorably, the contribution of the dam is either lost completely or delayed until she is retested with another sire. This is not necessarily the case in multiple sire mating (Figure 16). Potentially, the genotype of a dam may be tested in combination with the genotypes of several sires in the same litter, the progeny being compared as contemporaries.

It is proposed that by the use of multiple sire mating it is possible to produce a greater variety of gene combinations in a single generation than can be accomplished under single sire mating. This hypothesis is based on the feasibility of producing progeny in the same litter by more than one sire[3, 14]. It is further assumed that there are sufficient genotypic differences between sires within a single generation to justify the attempt to capitalize upon

these sire differences. Multiple sire mating should be a useful tool for gene recombination in small populations of swine to the extent that the above two factors are important. This implies radical use of existing genetic variability as has been proposed in plant breeding by Harlan[6].

The specific identity of the sire of an individual will remain unknown. However, lack of sire identity is not a handicap in a mass selection program because both parents are selected primarily upon their own performance.

Multiple sire mating does not readily lend itself to conventional procedures used in obtaining certain fundamental information, i.e., heritability estimates, genetic correlations, etc. The breeder must decide at the outset what information he wishes to obtain and what mating systems will be more suitable. Multiple sire mating studies can provide fundamental information with respect to methods of developing improved breeds and a better understanding of animal behavior as it may affect economic performance. If data regarding heritabilities and genetic correlations were sufficiently important to the progress of a project, new methods of estimation might be derived or parallel pedigree breeding could be undertaken to obtain such estimates.

Under multiple sire mating it may be impossible to reproduce a specific mating that may have led to the development of a superior individual. Yet it should be recognized that each fertilization is a unique event in recombination; the likelihood of extracting individuals with identical genotypic values by remating the same parents is extremely remote, particularly in view of the limited number of progeny ordinarily produced from an individual mating. Moreover, progress in the improvement of populations does not usually depend upon a single favorable recombination, but requires the production of large numbers of superior progeny.

Inbreeding and Relationship

In the development of improved breeds of swine (from outbred or crossbred foundations) by selection within closed herds, it is desired to raise the intra-herd relationship to a reasonably high level. The objective is to develop a "reproducible genotype" so that if repeated samples are drawn from the same population, animals of similar genotypic value can be obtained for linebreeding or crossbreeding. Multiple sire mating can contribute to this objective in two ways. First, the relationship between litters of relatively unrelated dams can be increased when sired by the same boar (this possibility exists under either multiple sire or single sire mating). In addition, the relationship between progeny of two unrelated sires can be increased if produced by the same dam.

Under multiple sire mating, the relationship between individuals within a population would tend to become increasingly similar with little opportunity for the permanent formation of genetic isolates because the population would always share a common gene pool. This is not necessarily the case under regular systems of mating. For example, in brother-sister mating the original population becomes subdivided into many families because each litter becomes an automatic genetic isolate. Without deliberate recombination of families, differences between litters within the breeding herd as a whole will tend to increase due to genetic drift. In fixed mating systems gene flow between families is prevented or must be regulated whereas under multiple sire mating this recombination occurs freely. Theoretically the results obtained by the two approaches might be quite different, but this consideration is beyond the scope of this paper.

Earlier inbreeding studies with swine have indicated that it is not possible to inbreed rapidly without a marked depression in performance[2]. Although there have been no extensive comparative studies of rates of inbreeding in swine, there is some evidence to suggest that a slower rate of "flexible" inbreeding accompanied by heavy selection pressure may allow the breeder to offset some portion of the expected decline caused by inbreeding effects[4]. This is supported by the more critical study of Tantawy[16] with *Drosophila*, and the classic rat experiment of King[8].

It has been pointed out that different rates of inbreeding have a different meaning in terms of their tendency to reduce genetic variance[7, 17] indicating the relative nature of inbreeding effects. There seems to be little reason to strive toward a particular coefficient of inbreeding or intra-herd relationship if this is to be attained only at severe sacrifice in economic performance. It appears more logical to retard the rate of inbreeding and, in the process, bring about fixation of only those gene combinations that are compatible with reasonably high economic performance. During long term breeding programs, it is likely that the number of loci that become homozygous due to the combined result of inbreeding and selection, will increase unless or until a condition of balanced heterozygosity is attained. It is possible that by the continual recombination of genes, new combinations may evolve in a closed population that will aid realization of a reproducible genotype without a marked reduction in performance. It is still an open question as to what level of homozygosity is essential in parent lines to insure uniform performance in crossbred combinations.

Multiple sire mating might be used to retard the rate of inbreeding, especially during the early generations of a breeding program when recombination of major genes will ordinarily be of greatest consequence. The rate of

inbreeding may be controlled by regulating the number of sires used for mating. In the initial phase of development from a wide genetic base, genetic differences between individuals will tend to be large, although these differences may not be obvious if widely different genotypes produce similar phenotypes. Therefore, to make more efficient use of existing variability it would be advantageous to use more sires relative to the number of dams. Gradually the number of sires used for mating might be reduced as the apparent genetic variation within the line is diminished. The level of genetic variance in this case might be more accurately determined by evaluating the variation in combinations of various superficial traits (e.g., head, ear and general body type, color etc.) than by changes in the variance of the economic traits studied. The basis for the statement is discussed by Sumption *et al.*[15]

Future Work

There are a number of questions that must be answered in order to assess the potential value of multiple sire mating in swine. Little evidence is available to determine the possible magnitude of the effects of natural selection for breeding efficiency among males or females. The genetic nature of variations in mating behavior are worthy of investigation in view of the breeder's interest in increasing selection pressure for this trait. It will be important to determine the number of boars that may be effective in siring progeny in a single litter, in relation to considerations of recombination. The effects of order and frequency of mating should be evaluated. The maximum interval that may elapse between two effective matings is not well established. The two latter questions are pertinent to the likelihood of a significant frequency of multiple paternity. Future experiments will attempt to shed light upon several of the above questions.

Summary

The use of multiple sire mating in the first five generations of development of the Minnesota No. 3 line of swine is described. Nine to 12 males were exposed continually as a group to 50 to 75 females during a single estrus cycle. General observations of breeding behavior were made.

The major reasons for adopting the multiple sire approach were to (a) induce natural selection for aggressive mating behavior, (b) augment simplicity of handling a larger population during the development of new lines.

Observations revealed a high frequency of mating and a definite but variable level of competition among sires.

Within-litter variation in color and type suggested the likelihood that a majority of the litters were sired by more than one boar.

The following potential values of multiple sire mating in swine are discussed: (a) natural selection, (b) increasing the diversity of the gene combinations produced within a single generation, (c) development of a "reproducible genotype" through maintenance of a large, single Mendelian population, and (d) retardation of the rate of inbreeding during early generations to afford "trial" of many unique gene combinations.

Further investigations will be reported in subsequent papers.

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TELEGONY RETESTED

(*Continued from page* 274)

and general body size and configuration were the obvious characters studied. Controls of related albino females were bred only to albino males for one to three litters.

Three hundred offspring were born. All those by pigmented males were pigmented. All subsequent offspring by albino males were albino and all offspring of the controls were also albino. The offspring of Sprague-Dawley females by hooded males were hooded like the father, but those from Wistar females by hooded males were pigmented over the entire body, except for a white strip on the ventral side. The pigmentation varied from dark gray agouti to black. This is explainable by noting that Sprague-Dawley albinos originated from hooded stock* and Wistar albinos originated from wild gray stock[3].

Three different genetic strains of *Drosophila* were also cross-bred in much the same way as the rats just described. Wild-type females were bred to males of two other strains, namely a black-body, vestigial wing strain and a yellow-body, white-eyed strain. Females of each of these were also bred to males of the two other kinds. These females were kept isolated from the males for 20 days after the egg-laying period to overcome the possibility of sperm retention from the first breeding. After 20 days the females were bred again to males of the same strain and the offspring checked through the F_2 generation. Controls were also used here, as before.

All hybrid offspring showed dominant characteristics, and all offspring of the second mating (identical male and female) showed pure strain characteristics through two generations. All control offspring were consistent with their strain.

In these experiments transmission of genetic traits was not accomplished through absorption of sperm by females, and the idea of telegony was not verified. The results furnish data to disprove telegony.

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HEREDITARY MULTIPLE SEBACEOUS CYSTS

F. E. STEPHENS*

OCCASIONAL reports of familial multiple sebaceous cysts (wens) appear in the literature. Some workers have considered the tendency to produce these cysts to be hereditary. Dominant, recessive, and sex-linked inheritance have been reported. Several workers have reported instances where sebaceous cysts have become malignant, or have been associated in some way with malignancy. We have studied recently a kindred at the Laboratory of Human Genetics at the University of Utah (Kindred 77) which shows a high frequency of sebaceous cysts. Our purpose was to discover the type of inheritance involved, and to determine whether or not the condition is related in any way to malignancy.

Description of the Trait

In Kindred 77 sebaceous cysts were found to occur in multiple form, varying in number from a few to several dozen, and ranging from the size of a pea to that of a walnut. While they were most frequently found near the hair line on the head and neck, they often occurred elsewhere on the body, such as the bend of the arm opposite the elbow. One case was reported of a cyst inside the nose. The age of onset varies greatly from the age of 10 to 40 years. When they first appear, they consist of a soft viscous-like material which later becomes hard and gristle-like. They can be removed and do not reoccur if the membraneous sack surrounding them is also removed.

History of Kindred

The occurrence of sebaceous cysts in Kindred 77 is shown in the pedigree chart in Figure 17. Kindred 77 consists of the descendants of I-1, who was born in England in 1807 and later emigrated to Utah. Two of his 10 children, II-7 and II-9, are known to have had sebaceous cysts. This characteristic has been found in five generations of this kindred. While in most cases the trait does not occur in the offspring unless it occurs in at least one of the parents, there are a few exceptions. Four individuals, III-1, III-6, IV-8, and IV-67, apparently do not have the trait, but have transmitted it to some of their children. It is possible that some of these were actually affected and for some reason it was not possible to secure the correct information. So far as is known, no cases of sebaceous cysts in this kindred have become malignant.

Literature

Paget (cited by Gates[3]) considered cysts were more commonly inherited than cancer. Paget[8], Klausner[5], Prakken[9] and Weber[11] (cited by Ingram[4]) all reported families showing familial multiple sebaceous cysts. Ingram and Oldfield[4] reported a family having five affected individuals in three generations. They concluded that "the factor for multiple sebaceous cysts appears to be inherited in a Mendelian fashion and resembles a dominant more than a recessive."

Monro[7] described a family in which 20 persons had sebaceous cysts. He explained this on a basis of a single Mendelian dominant, subject to modifications. He also concluded that there might be some relationship between sebaceous cysts and malignancy. Levit[6] reported a kindred showing 10 affected cases in three generations. He concludes that the trait is due to recessive inheritance. Sedgwick[10] (cited by Gates[3]) reported on two families showing sebaceous cysts. He considers the trait to be sex-linked.

Caylor[1] reported that "approximately 3.44 percent of the sebaceous cysts in this (his)

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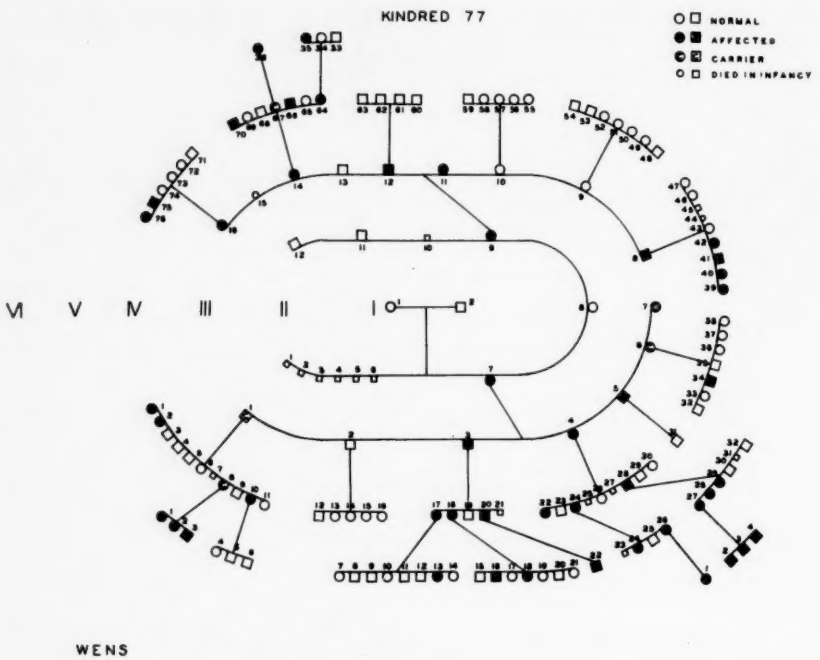

PEDIGREE OF KINDRED 77

Figure 17

The occurrence of sebaceous cysts has been found in five generations of this Kindred. It is concluded that it is inherited as a simple Mendelian dominant.

series eventually became malignant" and Gardner[2] reported sebaceous cysts to be associated with multiple polyposis in a syndrome, but found no cases where the cysts became malignant. Welch[12] reported a case where two different sebaceous cysts in the same individual became malignant.

Inheritance

While the inheritance of sebaceous cysts has been reported as being dominant, recessive, and sex-linked, most workers seem to agree on some type of dominant inheritance. It will be seen from the pedigree history of Kindred 77 that with four exceptions, the trait does not occur in a child unless it has also occurred in one of the parents. It also occurs approximately equally often in males and females. If we include the four exceptions mentioned above as carriers of the defective gene and classify the offspring of affected individuals into affected and normal, we get 47 affected to 56 normal which is a good approach to a 1:1 ratio which would be expected if we were dealing with a simple dominant trait. From the data which is available it seems that multiple sebaceous cysts in Kindred 77 are inherited as a simple Mendelian dominant with possible incomplete penetrance.

Summary

Forty-eight cases of sebaceous cysts or wens were found in four generations of Kindred 77. The trait occurs approximately equally often in males and females. With four exceptions it never occurred in a child unless it also occurred in at least one of the parents. It is concluded that it is inherited as a simple Mendelian dominant with possibly incomplete penetrance. There seems to be no relationship between sebaceous cysts and cancer in this kindred.

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GENETICS OF COAT COLOR IN HORSES

A Brazilian geneticist has recently published a comprehensive and critical review of the literature on the genetics of coat color in horses,* the most complete survey of the subject which has been made since the important publication by Odriozola in 1951. Where the views of Odriozola are at variance with those of North American geneticists, the author shows preference in general for the latter, but with minor deviations of his own. This publication will be of much interest to readers of the *Journal of Heredity*, where the more important studies in this field have been published or reviewed.

W. E. CASTLE

*Genetica da Pelagem do Cavalo. Professor Raul Briquet, Junior. Instituto de Zootechnia, Rio de Janeiro, Brazil. 115 pp., December, 1959.

A LETHAL GENE IN THE CASTOR BEAN PLANT

DAN ATSMON*

CASTOR BEAN MUTANT

Figure 18

A—Comparison of mutant embryo (top) and a normal one inside the dormant seed; while the cotyledons of the normal embryo cover nearly the whole surface of the opened seed, they occupy only a small fraction of it in the mutant. Note the normal size of the rest of the mutant embryo. (About ×1.5 of natural size.) *B*—Same as above, dissected out from soaked seed. Same enlargement as in *A*. *C*—Normal (extreme left) and mutant seedlings of the same segregating population after emergence. Mutant seedlings can differ in the extent of shrinking but none develops any further. (Reduced about ×3.)

THE annual-perennial nature of the castor bean plant (*Ricinus communis* L.), its extreme variability in all respects, the lack of crossing barriers between the most extreme types, and the long flowering season (practically all year round, under Israeli conditions) make it very favorable for genetic studies.

Still, not more than a few dozen genes were studied in this plant and except for one single case, (Patwardhan[6] and others) no linkage relationships have been established until now. Most of the characters which have been studied genetically until now are of a purely morphologic-qualitative nature. For further details concerning the genetics of this plant see Literature Citations[5, 7, 8, 12, 2, 10, 15 4]. Peat[8] has described "pink seedlings" of the castor bean plant; this character is controlled by a recessive gene.

Similar mutants to the one described here, although not always entirely lethal, were mentioned for corn and cotton and are probably found in many other plants. In corn such non-developing seedlings were described by Brunson[1], Demerec[3], Phipps[9] and others; especially the gm_1 described by Demerec[3] seems to be like the present case, characterized by a normal development of the endosperm but much reduced embryo which leads to lethality. In cotton a similar case was described by Yu[13].

Description of Mutant

The castor bean mutant described here is characterized by extremely shrunken and undeveloped cotyledons (see Figure 18). The rest of the embryo as well as the other parts of the seed (endosperm, seed coat and carun-

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cle) are normally developed. Such mutants do germinate, although somewhat later than the normals, but after emergence they never develop further; no initials of the first true leaves can be detected and sooner or later (some were kept in this state for weeks) they rot out, although they have perfectly developed hypocotyls and roots. The shrunken cotyledons never have the usual green color, but are pale-green with yellow-reddish necrotic areas. In those mutants where the character is expressed more extremely, there can be found just reddish rudiments of the cotyledons. There are no intermediates between the normal (whether heterozygous for this gene or homozygous dominant) seedling and the mutant one, and classification is very easy. In spite of many efforts, none of the mutants could be kept alive for an appreciable amount of time under greenhouse conditions. None of them showed any initiation of further growth.

The mutant was found among the inbred offsprings of a plant which was of hybrid origin. The hybrid nature of the original plant appears to have nothing to do with the occurrence of the mutant and it is assumed that it came about by a spontaneous mutation. In neither of the parental inbred lines has there ever been detected a similar mutant, although they have been used extensively for different genetic studies.

Since the original discovery of the mutant, it became evident that this lethal character is controlled by a single pair of recessive genes. Progenies of eight plants segregating for this character were analyzed (see Table I). In all cases the ratio of 3:1 between the segregants was very clear.

Some of the genetic data concerning this character are given in Tables I and II, as evidence of the simple genetic mechanism which underlies it.

Table I brings the data from four segregating populations, out of the eight analyzed for this character.

In some cases normal segregants of such populations were grown to maturity and their selfed progeny was examined for the occurrence of the mutant seedlings. In this way, normal homozygote dominants were differentiated from heterozygotes. Results of such progeny tests are given in Table II. In both cases deviations from the expected ratios (in the case of Table I—3:1 and in Table II—2:1) were well within the 95 percent of probability and thus there is no doubt as to the genetic mechanism of this character.

Summary

A lethal mutation in the castor bean plant (*Ricinus communis* L.) is described. The mutation is characterized by extremely shrunken cotyledons both in the embryo and in the seedling after emergence. No affected individual was seen to develop beyond the seedling stage. The character is controlled by a single pair of recessive genes.

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TABLE I. Results of classifications in populations segregating for the lethal gene "shrunken cotyledons"

Progeny no.	Total no. of seedlings	No. of affected (mutant) seedlings	No. of normal seedlings
759-1	79	17	62
759-3	85	17	68
759-6	63	18	45
759-7	98	24	74

TABLE II. Results of progeny tests done in some of the normal segregants (last column in Table I)

Progeny no.	No. of progeny tests	No. of dominant homozygotes (breeding true for normal seedlings)	No. of heterozygotes (segregating for shrunken cotyledons)
759-1	40	12	28
759-6	27	8	19

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